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OBSERVATIONS ON THE TOXIN OF *RHIZOPUS NIGRICANS*

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Introduction

In a preliminary note [1] we have shown that the "presssaft" from *Rhizopus nigricans* contains a powerful toxin. At that time we had not attempted to prepare the toxin in a more concentrated form and had only ascertained that an aqueous extract from 0.045 gram of the dried mould filaments or mycelium was sufficient to kill a 1.35 kilo rabbit in two minutes when the toxin was injected intravenously.

In this paper we desire to present some further observations on the chemical nature of this toxin, methods of obtaining a more concentrated preparation and the results of physiological tests. Although we believe that we have not, as yet, isolated the toxic principle in a pure state, we have obtained preparations having a lethal toxicity of approximately 1:275,000 parts of body weight when injected intravenously into rabbits. Inasmuch as one of us (G.) must of necessity abandon further work on this subject we are presenting the results more recently obtained.

Experimental

1. *Physiological Tests using Extracts of the Mycelium or Aerial Filaments*

(*a*) **Intravenous Injections.**—The presence of a toxin in the "presssaft" of *Rhizopus* was noticed during a series of immunization experiments having as their aim a chemical analysis of the cause of sex in the Mucors.[2] The aerial filaments of the mould were ground with sand and the juice expressed by means of a small beef juice press and centrifuged at high speed to remove suspended solids. When 2 c.c. of such an extract is injected intravenously into the ear of a rabbit death is almost instantaneous, the animal often expiring before the injection is complete (about 5 seconds) or before the needle can be withdrawn from the vein. In such a case there are no typical symptoms accompanying death, there is rarely any struggle and only a sudden sinking of the head to one side and a convulsive twitching of the body.

When, however, only about *one tenth* of this dose is given (diluted to 2 or 3 c.c. with physiological salt solution) the symptoms are invariably the same. A few seconds after the needle is withdrawn the head sinks to one side and a violent convulsive spasm follows. This may be followed by a "typical death" (see below) or by a short rest period followed by another convulsive spasm, depending both on the size of the dosage employed and also on the degree of resistance possessed by the animal.[1] The convulsive spasms rarely last longer than 30 seconds while the rest period between may be as long as 30 minutes. During the rest periods the animal usually lies fully stretched out, the only indication of life being its regular breathing. At the close of the final spasm there is usually a short rest period of 5 to 10 seconds followed by a spasmodic cough-like movement of the diaphragm with widely opened mouth as if gasping for breath. There are usually 8 to 10 of these cough-like movements of the diaphragm, each shorter and more rapid than the one preceding. During this period the head

[1] We have observed considerable individuality in these animals; in some instances one animal would be killed almost instantly while with the same dosage another animal of like weight would live for 15–20 minutes.

is thrown backward and the chest and abdomen are distended and turgid, so much so that only by strong pressure can the wall of the abdomen be flexed. This reaction usually lasts 8 to 12 seconds and is followed by a relaxation of the rigid abdomen, protruding eyes and death. This description of a death is meant wherever a "typical death" may be referred to later. All of these symptoms may be shown within a period of 15 seconds from the time the injection is made or death may be deferred for several hours. In the latter case the behavior of the animal is usually "normal" until within a few minutes before death. Such a condition accompanies a dosage *just sufficient* to cause death.

When the dosage is *just sub-lethal* there is usually a short convulsive spasm followed by a long period (24 to 48 hours) of extreme lethargy, and complete recovery. In nearly all instances tested a fall of nearly 4° in the body temperature of the rabbit occurred during the first 12 hours after receiving the injection, and as recovery progressed the temperature gradually rose. Since the rabbit has no constant temperature it is impossible to determine the significance of this factor with exactness, but it seems probable that a fall from 102.4° to 98.5° is significant, and such a fall has been observed in several instances.

No tolerance was observed when an animal which had received a just sub-lethal dose was again given a similar injection after complete recovery. On the contrary the symptoms were slightly more violent than in the first injection.

When a *slightly* smaller dose than just sub-lethal is injected *there is usually no noticeable reaction*, the animal remaining to all appearances normal. If, however, after 30 to 60 minutes, a slight additional quantity is injected *just sufficient to raise the original dosage within the lethal limits*, there is a sudden violent reaction and death within a few seconds or minutes.

(*b*) **Subcutaneous Injections.**—Only four experiments were made where the "presssaft" was injected subcutaneously, but since each of these injections gave similar results it was thought unnecessary to prolong the investigation further. In each instance the injection was made under the skin of the back under aseptic precautions. The "presssaft" was sterilized either by rapidly heating the solution to *just boiling* and then cooling quickly or else by the addi-

tion of ether or chloroform in a sterile tube, plugging with sterile cotton and evaporating the antiseptic under diminished pressure.

A typical instance of the results is as follows: Rabbit No. 60, weight 1330 grams, received subcutaneously 6.7 c.c. of the "presssaft" at 11 A.M. May 21. This dosage was the equivalent of about 20 intravenous lethal doses. No results were observed during the first 24 hours. After several days it was noticed that the skin seemed loose and the flesh "flabby" but no other symptoms were observed. Eight days after the injection a small purulent ulcer was observed on the belly of the rabbit and the weight had fallen to 1050 grams. Although the ulcer was well washed with "peroxide" and bound up with iodoform gauze it increased in size and inasmuch as it showed no sign of healing the animal was chloroformed 12 days after making the injection. The weight at death was 850 grams.

This result would seem at first glance to be due to bacterial infection at the time the injection was made, but we do not believe this to be the case, for in numerous instances we have injected subcutaneously the "presssaft" of other moulds (as well as physiological salt solution) with far less precaution against infection and have never had such injection followed by either lesions or by a considerable loss in weight.

On examination we found that there was an open channel from the site of the sore to the point of injection and subsequent investigation in other cases showed that the "presssaft" is not absorbed (or else that serum exudes) so that the lump formed by the mass of liquid under the skin gradually works down the side of the animal until it reaches the belly. This movement of the liquid under the skin is probably brought about by the action of gravity, possibly combined with a corrosive action of the toxin. After reaching the belly the mass of liquid can be distinctly felt under the skin for 2 to 3 days, at the end of which time the skin is eaten through from within and the ulcer begins.

(*c*) **Administration of the Mould per Os.**—Rabbits, guinea pigs, and chickens were fed the moist mycelium or the dried and ground mycelium and aerial filaments of *Rhizopus*. In only one instance was there any noticeable effect. A guinea pig weighing 705 grams

was fed one gram daily of the dried mould mycelium in gelatin capsules on five consecutive days. There was a marked loss of weight each day and at the end of the sixth day the weight had fallen to 505 grams, showing a loss of nearly 30 per cent of the original body weight. Unfortunately at about this time one of the contagious diseases to which guinea pigs are subject developed in the animal house and in the course of the next 10 days all of the pigs had died. We are unable, therefore, to say with certainty that this loss in weight was due to the feeding of the mould although we believe that such may be the case, since the animal was apparently perfectly normal in its actions during the feeding experiment and also since it was one of the last of the animals to die.

Rabbits were fed upon bread upon which a heavy culture of the mould was growing and also upon corn meal upon which *Rhizopus* had been grown for a week or more. No ill effects of such feedings were observed.

2. *Autopsy after Death following an Intravenous Injection*

Dr. O. T. Avery of the Hoagland Laboratories, Brooklyn, was kind enough to witness the symptoms caused by an intravenous injection of the toxin into both rabbits and guinea pigs and also to assist in an autopsy. The only abnormal conditions apparent were a pronounced heart block, the auricles beating about 3:1, and a partial inflation of the lungs. Avery has investigated the effects of the toxin prepared from the tubercle bacilli by the method of Vaughan [3] as well as the toxic properties of the split products of edestin [4] and he pronounced the death symptoms and the autopsy following *Rhizopus* injection as being "typical" of toxins of this type, and practically indistinguishable from an anaphylactic intoxication. Unless we assume that all animals are born "sensitive" to *Rhizopus* it will be almost impossible to call the death anaphylaxis. On the other hand the preparation of the toxin precludes any splitting of the protein molecules such as is brought about by Vaughan's hydrolysis with alkali. It seems possible however that *Rhizopus* so utilizes the protein of the culture medium that a split product having toxic properties similar

to the toxins prepared by Vaughan's method is present in the "presssaft."

3. *Attempts to Isolate the Chemical Substance Responsible for the Toxic Action*

About 5 c.c. of the "presssaft" was evaporated to dryness *in vacuo* over sulphuric acid and extracted first with warm ether and then with warm alcohol. The ether and alcohol were evaporated in a dessicator and the residue taken up with physiological salt solution and injected intravenously into a rabbit. No ill effects were observed. It was later found that the toxic principle could be completely precipitated from its aqueous solution by the addition of four volumes of 95 per cent alcohol. Following this observation a concentration of the toxin was obtained by two methods.[1]

Method. (*a*)—The mycelium of *Rhizopus*, grown on solid agar medium under conditions of extreme moisture with a consequent nearly complete suppression of sporangial formation, was stripped

[1] Since preparing this paper for publication our attention has been called to the work of Paladino-Blandini.[5] Paladino-Blandini tested *Rhizopus*, together with other genera, for the presence of a toxic phenol by extracting the dried mycelium with 90 per cent alcohol evaporating the extract to dryness, and injecting a suspension of the dried residue. In *Rhizopus* three injections of 0.05 grams each, spaced at an interval of half an hour, were necessary to kill a 3 ko. rabbit. The death symptoms of the rabbit were very similar to those which we have observed with our toxin. We have, however, injected 0.108 gram of the alcohol soluble substances (extracted from the aerial mycelium with *absolute* alcohol in a Wiley extraction apparatus) into a 1200 gram rabbit without ill effects. It seems possible that extraction with 90 per cent alcohol may dissolve a part of our toxin, or, which seems still more probable, that some of the toxin formed a colloidal solution with the 90 per cent alcohol. This will occur whenever alcohol is added to a solution of the toxin in the absence of electrolytes. Another possible cause of death is embolism due to the very considerable quantity of insoluble lipins injected into the vein.

It is certain that Paladino-Blandini had only a small part of the toxin present in his *Rhizopus* mycelium, for we have found that the alcohol soluble material does not exceed 30 per cent of the dry weight of the fungus, therefore to obtain 0.15 gram of the alcohol soluble portion would require 0.50 gram of the dry mycelium, the aqueous extract of which would contain sufficient toxin to kill *five* 3 kilo rabbits *within two minutes* after the injection.

off, cleaned from agar, dried over sulphuric acid *in vacuo* and powdered.[1]

The powdered mould in 25 gram lots was poured into 200 c.c. of boiling water and stirred for about 30 seconds, then filtered on a Buchner funnel with suction. This process was repeated on the residue twice more with fresh hot water, the filtrates were united and concentrated at 50° to 55° under a pressure of about 30 mm. to about 200 c.c. This solution was then placed in a collodion bag and dialysed against repeated changes of distilled water, or, since tests showed that the water outside the bag contained no toxin, against running water for 72 hours, using chloroform as an antiseptic inside of the bag. The liquid in the bag was clear, straw-colored and opalescent, it frothed quite readily and dripped fairly viscid. This liquid was centrifuged at high speed to remove some flecks of solid (agar?) and was then poured into four volumes of 95 per cent alcohol. A heavy white precipitate formed at once. The precipitate was allowed to settle in a tall cylinder, the yellowish supernatant liquid was siphoned off and the white solid packed in a tube by centrifugation. The solid was stirred with fresh alcohol, again packed in a centrifuge and dried over sulphuric acid *in vacuo*. Yield 4.85 per cent from the ♀ race and 4.60 per cent from the ♂ race.[6]

Method. (*b*)—One hundred grams of the dried and ground mycelium was stirred into 2000 c.c. of water at the room temperature and allowed to stand with frequent stirring for two hours. The yellowish liquid was then filtered by suction, the residue pressed strongly in a large beef juice press and the liquid obtained added to the first filtrate. Three volumes of 95 per cent alcohol were then added to the united filtrates, yielding at once a copious white precipitate. The partially extracted mould was again stirred into 1000 c.c. of water and allowed to stand at room temperature for 18 hours, when the mixture was filtered by suction and the residue

[1] That a possible slight admixture of the nutrient medium with the mycelium has no significance so far as the toxin obtained is concerned, is shown by check experiments with the fungus grown on oatmeal where all possibility of contamination with the substratum was avoided by carefully cutting off for use only the free aerial mycelium. This preparation showed a toxicity which was *possibly* slightly greater than that of the mycelium from agar.

again pressed dry. This filtrate was likewise precipitated by three volumes of alcohol, yielding a small but appreciable amount of precipitate. The alcoholic solutions were united in a large vessel and allowed to settle for 48 hours, the clear yellow supernatant liquid was then siphoned off and the white solid filtered on a Buchner funnel with suction. The solid was air dried and dissolved as completely as possible in 250 c.c. of water *without the aid of heat.* A considerable quantity did not dissolve (coagulated albumen [?] or agar [?]). The solution was then centrifuged and the clear amber liquid poured into 1000 c.c. of alcohol. The precipitate was allowed to settle, the solution centrifuged and the white solid dried *in vacuo* over sulfuric acid. This solid was powdered and again dissolved in 150 c.c. of water, only a few particles remaining undissolved. The mixture was allowed to stand for 12 hours, centrifuged and poured into 1000 c.c. of alcohol. A white turbidity resulted but on standing for 24 hours no precipitate settled. This was probably caused by an almost complete removal of electrolytes from the solution, for on the addition of two drops of a saturated solution of sodium chloride a copious curdy precipitate was at once thrown down, leaving the supernatant liquid perfectly clear and colorless. This precipitate was packed in a centrifuge, dried *in vacuo* over sulphuric acid and powdered. Yield, 3.58 per cent from the ♀ race and 3.74 per cent from the ♂ race of the mould.

4. *Physiological Tests with the Concentrated Toxin*

(*a*) **The Intravenous Lethal Dose.** — The data for this series can be best expressed in the form of a table, the various preparations of the toxin being designated by (*a*) ♂ (prepared by method [*a*] from the ♂ race of *Rhizopus*) (*a*) ♀, (*b*) ♂, and (*b*) ♀.

(*b*) **Subcutaneous Injection.** — Only one animal was given a subcutaneous injection of the concentrated toxin. Inasmuch as we have found that sterilization with heat causes a loss of toxicity (see "Chemical Properties of the Concentrated Toxin," below), the dry powder was sterilized by placing it in a sterile vial, adding about 5 c.c. of a mixture of ether and 95 per cent alcohol, plugging the vial with sterile cotton and, after allowing the preparation to

Toxin	Wt. injected	Vol. of injection	Wt. rabbit	Time to die	Toxin to body wt.
(*a*) ♂	0.0080 gr.	8 c.c.	1050 gr.	50 mins. "typical"	1: 131,250
	0.0060 gr.	6 c.c.	875 gr.	3 mins. "typical"	1: 145,800
	0.0025 gr.	2.5 c.c.	560 gr.	90 secs. "typical"	1: 224,000
	0.0040 gr.	4 c.c.	1000 gr.	Recovered	1: 250,000
(*a*) ♀	0.0080 gr.	2 c.c.	1000 gr.	19 mins. "typical"	1: 125,000
	0.0040 gr.	1 c.c.	1000 gr.	Recovered	1: 250,000
(*b*) ♂	0.0065 gr.	3.3 c.c.	1390 gr.	5 mins. "typical"	1: 215,000
	0.0050 gr.	2.5 c.c.	1365 gr.	Nearly normal for first 5 hrs. but found dead at 18 hrs.	1: 273,000
(*b*) ♀	0.0080 gr.	4 c.c.	1200 gr.	60 secs. "typical"	1: 150,000
	0.0075 gr.	3.75 c.c.	1510 gr.	75 secs. "typical"	1: 200,000
	0.0072 gr.	3.6 c.c.	1550 gr.	3 mins. "typical"	1: 215,000
	0.0070 gr.	3.5 c.c.	1535 gr.	1 min. 40 secs. "typical"	1: 220,000
	0.0065 gr.	3.25 c.c.	1475 gr.	5 hrs. "typical"	1: 225,000
	0.0036 gr.	1.80 c.c.	810 gr.	Not dead in 7 hrs but dead in 15 hrs.	1: 225,000
	0.0040 gr.	2.0 c.c.	1480 gr.	No typical symptoms. Recovered	1: 370,000

stand for several hours, evaporating the alcohol-ether in a dessicator over sulfuric acid. The dried residue was then dissolved in sterile salt solution.

Under aseptic precautions 0.10 gram of preparation (*b*) ♀, dissolved in 5 c.c. of physiological salt solution, was injected subcutaneously on the back of a rabbit weighing 1550 grams. No immediate effect was observed. Four days later, however, the weight had fallen to 1300 grams and the flesh was very flabby and soft. There was a soft lump low down on the side of the animal and immediately below the point of injection. This lump evidently contained serum or the unabsorbed liquid which was injected. No

sore had as yet opened. The following day, however (five days after the injection) there was a small ulcer at the spot where the lump had been the previous day. The sore continued to spread somewhat, but contrary to the experiments where the "presssaft" was injected, a scab formed over the sore. This scab or thickened skin area could be distinctly felt as a heavy ridge extending from the point of injection to the spot where the skin had broken through. Following the formation of the scab there was a decided improvement in the condition of the animal and 13 days after the injection the weight had risen to 1530 grams. Following this the scab became loosened, leaving the flesh dry. There was no pus formation observed. The animal increased in weight and the lesion healed normally.

(*c*) **Intraperitoneal Injection.** — Two experiments were made in which the concentrated toxin was injected into the peritoneal cavity, using preparation (*b*) ♀.

Rabbit No. 1 weighed 1440 grams and received 1.5 c.c. of a solution containing 0.06 gr. of the toxin, or 1:24,000 parts of body weight. The animal appeared normal for four hours following the injection but was found dead 12 hours later. An autopsy showed that the lower intestine was highly inflamed and that the peritoneal cavity contained a considerable quantity of a yellow fluid, the lungs were discolored with gray blotches but the other organs were normal.

Rabbit No. 2 weighted 1625 grams and received 2.5 c.c. of a solution containing 0.10 gr. of the toxin, or 1:16,250 parts of body weight. No unusual symptoms were observed for some time with the exception that the animal did not seem as active as usual. Forty-eight hours later the weight had fallen to 1450 grams, the temperature was 104°, the animal had diarrhoea and was running at the nose and the skin of the abdomen was highly inflamed. Ninety-six hours after the injection the animal was found dead, weight 1360 grams. An autopsy showed the caecum filled with a highly putrescent liver-colored mass, the stomach packed with food, while the lower intestine and the rectum contained only four or five formed feces whereas normally there is a large number. The small intestine contained a small quantity of a thin yellow fluid (probably bile) and was very much distended with gas. The gall

bladder was three or four times normal size and the liver, otherwise normal, was badly stained with bile. The spleen was much darker than normal, being almost black at the median end. The lungs were a blackish gray, badly mottled, without a trace of pink color being apparent. There was no evidence of inflammation within the body cavity.

(*d*) **Intramuscular Injection.** — Only one experiment was made where the injection was given intramuscularly, and this injection was not an intentional one. A rabbit weighing 1310 grams was given an injection of 5 c.c. of a solution containing 0.20 grams of preparation (*b*) ♀. The intention was to give the injection intraperitoneally, but later developments and the autopsy showed that the needle had not penetrated the abdominal wall and that the injection had been between the muscle plates of the abdomen. Forty-eight hours after the injection the weight had fallen to 1180 grams, the temperature was 104.9°, the skin of the abdomen was highly inflamed and very tender to the touch, but no sores were observed and the animal ate regularly. Eight days after the injection the weight had fallen to 1045 grams and a bad purulent ulcer had formed on the abdomen. The animal was chloroformed and an autopsy performed. All of the viscera were in a normal condition and only a slight inflammation was observed on the inner wall of the abdomen immediately under the ulcer. In this instance, therefore, an intramuscular injection caused the same developments as a subcutaneous injection, only the symptoms were somewhat more pronounced. This may be due to the higher dosage of toxin (30 to 40 intravenous lethal doses) employed, or it may be due to the fact that the irritation was between the muscle plates rather than subcutaneous.

(*e*) **Administration of the Concentrated Toxin per Os.** — Only one feeding experiment was made but the quantity of the toxin was sufficiently large to determine whether or not the mould would be dangerous in moderate doses.

A rabbit weight 1330 grams was fed, by means of a catheter inserted into the stomach, 50 c.c. of a solution containing 1.0 grams of preparation (*b*) ♂ — in other words 150 to 200 times the intravenous lethal dose. *This is the equivalent of 26 to 27 grams of the dried mould mycelium and of probably 100 to 125 grams of the*

moist growing mould. It is a far larger amount than any animal could possibly procure at any one feeding under any natural conditions. No abnormal effect has been observed to date. The weight of the animal steadily increased, the animal weighing 1590 grams 19 days after the feeding.

From this experiment, in which we are certain that no loss of the toxin occurred through vomiting, we conclude that, in rabbits at least, the toxin is not absorbed from the alimentary canal, or if absorbed it has been so changed as to produce no ill effects.

5. *Chemical Properties of the Concentrated Toxin*

The product obtained by either method (*a*) or (*b*) forms a yellowish white powder readily soluble in water to a straw-colored opalescent solution from which it is again precipitated by basic lead acetate, phosphotungstic acid + HCl (without the addition of the HCl only a white opalescence is produced), three or four volumes of alcohol, and by mercuric chloride. Tannic acid gives a fine white precipitate with a solution of the toxin, and Folin's "Phenol Reagent" gives a deep indigo-blue color.

The Biuret, Xanthoprotein, Adamkiewicz's, Liebermann's and Molisch's tests are all strongly positive, while Millon's test is positive but faint. The substance does not reduce Fehling's solution, but after hydrolysis with acids a strong reduction takes place. The toxicity of the solution is not altered by passing through a Berkefelt filter, but on warming with freshly ignited animal charcoal all of the toxin is absorbed by the charcoal and the solution is absolutely non-toxic. This is still the case when the solution + bone black is quickly raised to *just boiling* and then rapidly cooled.

Digestion at 37° with pepsin in 0.2 per cent HCl for three hours did not impair the toxicity, but a further digestion of this solution with trypsin in a weakly alkaline medium for 18 hours (no antiseptic) caused a loss of about 75 per cent of its activity.

The toxicity is slowly destroyed by boiling. 0.20 grams of preparation (*b*) ♂ was dissolved in 50 c.c. of physiological salt solution and boiled under a reflux condenser for three hours. There was no evidence of decomposition nor was there any precipitate formed, but on testing the physiological activity of the solution it was found that the lethal dose was about 1:37,000 parts of body

weight. This would indicate the loss of about 75 per cent of the original activity.

When, however, the toxin is boiled with N/10 HCl there is a rapid loss of activity. 0.20 grams of preparation (*b*) ♂, was boiled under a reflux condenser with 25 c.c. of N/10 HCl for $1\frac{1}{4}$ hours, 25 c.c. of N/10 NaOH was then added and the solution, which now gave a strong reduction with Fehling's solution, was poured into four volumes of 95 per cent alcohol. The white precipitate which was thrown down was collected in a centrifuge, dried, dissolved in a small volume of physiological salt solution and injected into a rabbit intravenously. The dosage was equivalent to 1:14,000 but no ill effects were observed, indicating that all or nearly all of the toxin had been destroyed.

The possibility that we were dealing with a saponine was tested by adding a strong solution of the toxin to washed blood corpuscles, but no trace of hemolysis occurred.

Analyses

Nitrogen was determined on the different preparations in part by Dumas' method and in part by Kjeldahl's. From the nitrogen figures it is seen that we are not dealing with a pure compound but with a mixture, and therefore, no further analyses were attempted. From the figures for nitrogen and from the chemical tests to which the toxin responds one can postulate all sorts of classifications, such as glucoside, the split product of a protein, peptides, etc., but we are unwilling to even venture a guess as to the chemical nature of the toxin. That can only be determined when some method has been devised by which the toxic principle can be obtained in a pure condition.

Preparation (*a*) ♂:
0.1916 gr. gave 7.4 c.c. N at 24° and 768 mm. Nitrogen found = 4.43%.

Preparation (*a*) ♀:
0.2300 gr. gave 5.4 c.c. N at 22° and 768 mm., 0.4180 gr. gave 9.0 c.c. N/10 NH_4OH. Nitrogen found = 2.69% and 3.01%.

Preparation (*b*) ♂:
0.2554 gr. gave 10.9 c.c. N at 20.5° and 762 mm., 0.2260 gr. gave 9.6 c.c. N at 21.5° and 768 mm. Nitrogen found = 4.88% and 4.87%.

Preparation (*b*) ♀:
0.2977 gr. gave 10.25 c.c. N/10 NH_4OH, 0.2253 gr. gave 9.0 c.c. N at 23° and 767 mm. Nitrogen found = 4.82% and 4.54%.

6. *Evidences of a Toxin in Other Species of the* Mucorineae

During a series of injections we have obtained some evidence that there may be a small amount of a toxic substance in the "presssaft" of other moulds, such as "*Mucor V*" and *Phycomyces nitens*. We have made a large number of intravenous injections of the "presssaft" of these species and have observed that where a non-lethal dose is increased in an injection three to four days later death may occur. In two sets of experiments we have lost four out of five rabbits injected with the ♀ race of *Mucor V* while none of the animals receiving an equal quantity of the "presssaft" from the ♂ race have died. It was also observed that whereas 2 c.c. of the "presssaft" from the filaments of *Phycomyces* caused no untoward symptoms, that an injection of 3 c.c often caused convulsions and sometimes death. An attempt was therefore made to concentrate the toxin from *Phycomyces*, if such a toxin existed.

Twenty grams of the dry aerial filaments of the ♂ race of *Phycomyces nitens* was treated as in method (*a*) (page 358). The alcohol precipitate was dark green and the powder on drying was almost black. A solution of the dried preparation was too dark to permit of the observation of color tests. A solution of the powder did not reduce Fehling's solution, but after acid hydrolysis a strong reduction was observed. A yield of 2.60 per cent of the dried product was obtained.

In physiological tests no effect was observed when an intravenous injection of 1:15,000 was given. This preparation has therefor little or no toxic properties.

The fact that *Phycomyces* gives a yield of 2.60 per cent of a non-toxic substance by the same method that *Rhizopus* yields 4.60 and 4.85 per cent of a toxic substance confirms to our mind the fact that our toxin is far from being a pure compound. By the method of preparation glycogen, or glyco-proteins, would, if present, be precipitated with the toxin and would account for the Molisch's and Fehling's tests.

Summary

1. *Rhizopus nigricans* contains a powerful toxin which is water soluble and which can be completely precipitated from its aqueous solution by three to four volumes of 95 per cent alcohol.

2. A solution of the toxin is non-dialyzable, gives the protein tests as well as Molisch's test, but reduces Fehling's solution only after a previous hydrolysis with acid. The toxin is completely absorbed from its aqueous solution by warming with bone black, is slowly destroyed by boiling its aqueous solution and rapidly destroyed by boiling with N/10 HCl. Peptic digestion for three hours is without effect on the toxicity.

3. In intravenous injection into rabbits the lethal dose of our preparations lies between 1:225,000 and 1:275,000 parts of body weight.

4. In subcutaneous and intramuscular injections the toxin causes the formation of purulent ulcers.

5. In intraperitoneal injections there is some evidence that paralysis of the digestive tract ensues.

6. Administered *per os* in large doses there was no harmful effect observed.

7. Inasmuch as a 2.6 per cent yield of a *non-toxic* substance having similar chemical reactions can be prepared from *Phycomyces nitens*, and inasmuch as the nitrogen content of our toxic preparations varies from 2.8 per cent to 4.9 per cent, we believe that our preparations are, in all probability, highly impure, perhaps containing 50 per cent or more of inactive impurities, so that the actual intravenous lethal dose of the pure toxin is probably much higher than has been observed, possibly near the extreme toxicity of 1:500,000.

Literature

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EXPERIMENTS ON THE ORIGIN AND PROPAGATION OF THE IMPULSE IN THE HEART

IV. The Effect of Vagal Stimulation and of Cooling on the Location of the Pacemaker within the Sino-auricular Node.[1]

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IN a recent paper[2] we have shown by using the method of initial electric negativity to locate the origin of the heart beat, that stimulation of the vagus nerve might remove the seat of impulse formation from one part of the mammalian heart to another. So far as could be determined by means of initial negativity the pacemaking function always remained in some portion of the specialized tissue. Not only might the pacemaker migrate from the sinus region to the auriculo-ventricular node, but as first demonstrated by Zahn[3] it might be restricted in its location to certain parts of the latter. This we confirmed, and in one case we were able to convert a coronary sinus rhythm into an auriculo-ventricular one by vagal stimulation.

The discovery that the auriculo-ventricular node need not function as a whole but that a part may act as the seat of impulse formation suggested at once that the sino-auricular node be investigated along similar lines. Such an investigation, however, was brought to mind not only by this previous work on auriculo-ventricular rhythm but also by certain curves we had obtained on comparing various parts of the sulcus terminalis with each other.

[1] The preceding papers of this series have appeared in Heart, 1914, v, Nos. 2 and 3.

[2] Meek and Eyster: Heart, 1914, v, No. 3.

[3] Zahn: Archiv für die gesammte Physiologie, 1913, cli, p. 247.

Usually an electrode could be placed on a point which histological examinations had taught us was near the head of the node with assurance that this point would precede all others along the sulcus terminalis in negativity. At times, however, the area of initial negativity was lower than expected. A possible explanation was that the node was not functioning as a whole but that in these cases the lower part was acting as pacemaker. Experiments testing this idea seemed of value not only of themselves but for the light they might throw on the mechanism of vagus action and on the gradual shortening of the As-Vs interval which is often observed during the appearance and disappearance of auriculoventricular rhythm.

There is no experimental evidence which shows how small an amount of automatic tissue may function as pacemaker. The sinus node is, however, of considerable size and a division into functional parts seems quite possible. According to careful measurements by Lewis, Oppenheimer and Oppenheimer [1] the sinus node in 7 dogs averaged 13.7 mm. in length and 2 mm. in width. Koch [2] found the node 7 mm. long in a rabbit's heart. In 4 dogs' hearts examined histologically by ourselves the nodes averaged a trifle over 15 mm. in length. The sinus node is then of sufficient size to allow an analysis into parts by the electrical method of initial negativity.

Experimental Methods

In our first series of experiments the upper, middle and lower parts of the sinus node were compared with each other by means of the string galvanometer before, during and after periods of vagal stimulation. Non-polarizable electrodes were used which were attached to the heart with pieces of woollen yarn, the latter being stitched to the epicardium by a fine thread. This means of attachment insured a constant contact during all parts of the cardiac cycle. With suitable keys any of the points to be studied could be connected through the galvanometer and a photographic record of the movement of the string made on bromide paper with a long roll photographing apparatus. Precedence in activity

[1] Lewis, Oppenheimer and Oppenheimer: Heart, 1910–11, ii, p. 147.

[2] Koch: Medizinische Klinik, 1912, viii, p. 108.

was then determined by the direction of the auricular wave. To identify this wave the mechanical contraction of the auricle was also recorded by air transmission to a Marey tambour.

Although these experiments were positive, showing that during vagal stimulation the lower part of the sinus node might precede the upper in activity, it was felt that the results were not conclusive evidence of the shift of the pacemaker within the sinus node itself, since the beats at this time might have been arising in the auriculo-ventricular node, a condition which might show the lower part of the sinus node negative before the upper. To meet this objection it seemed necessary to compare the upper part of the sinus node with the lower, and the sinus node with the auriculo-ventricular node simultaneously. With the aid of two galvanometers this has now been done and it is these experiments that we wish to report at this time.

As mentioned before, three non-polarizable electrodes were placed as well as could be judged on the upper, middle and lower parts of the sinus node. To reach the auriculo-ventricular node a long curved glass electrode was passed down the external jugular vein and its end adjusted against the auricular septum just above the middle tricuspid valve. The middle sinus and auriculo-ventricular electrodes were now connected through the first galvanometer and the upper and lower sinus electrodes through the second. The electrodes were so connected that upstroke on the photographic curve in each case represented primary activity of the first member of the couple compared. In order to identify positively the auricular wave the mechanical contraction of the right auricle was recorded by means of air transmission. A signal to show the beginning and end of procedures and time in one-fifth seconds were also registered on the records. All the above were recorded on a single record, bromide paper of 12 cm. width being employed for this purpose.

Dogs were used in all experiments. The animals were morphinized, the chest opened and the heart exposed under ether anesthesia. The excellent artificial respiration apparatus recently described by Gesell and Erlanger[1] was used. To this was added an electric heating coil which was thrown into circuit by a thermo-

[1] Gesell and Erlanger: This journal, 1914, xxxiii, p. 33.

regulator and relay. In this way the air delivered from the tank was maintained at 55° C. and the mixture leaving the ether bottle was kept at approximately 37° C. With this slight modification of the Gesell-Erlanger apparatus the difficulties and inconveniences of artificial respiration seem almost entirely overcome.

The hearts were removed after the experiment and preserved in formalin. The position of the auriculo-ventricular electrode was carefully noted and in the first experiments histological examinations of the sinus region were made. In all hearts examined the position of the electrodes was either on or near enough the part desired to justify our conclusions. In the latter experiments the hearts have not been studied histologically but the electrodes were placed with extreme care and judging from the fairly constant position of the sinus node we feel certain they were either on or near the ends of the sinus node.

The vagi were stimulated with tetanizing currents from a Harvard induction coil. The most desirable strength of stimulus was found to be one that only slightly showed the heart. Pencils of ice and ethyl chloride sprays were used to cool the sinus node.

Experimental Results

1. *Vagal Stimulations.* — Three experiments were carried out in which comparisons of the upper sinus region with the lower, and of the sinus node with the auriculo-ventricular node, were made simultaneously during vagal stimulation. Thirty records were taken in 19 of which the right vagus was stimulated and in 11 the left. The following are the most important results obtained by a study of these records.

In 10 of the 30 records instances were found either of single beats or series of beats in which the upper curve comparing sinus and auriculo-ventricular node remained unaltered in direction, while the lower curve comparing the upper and lower parts of the sinus node had reversed. Fig. 1 illustrates a case of this kind. In the first two cycles of this record the auricular wave of each curve begins with an upstroke, indicating that the sinus node preceded the auriculo-ventricular node (upper curve) and that the upper part of the sinus node was active before the lower

(lower curve). In the third cycle at the end of a two-second period of left vagal stimulation the upper curve remains the same in direction, but the auricular wave of the lower curve has reversed. This indicates that while primary activity was still in the sinus

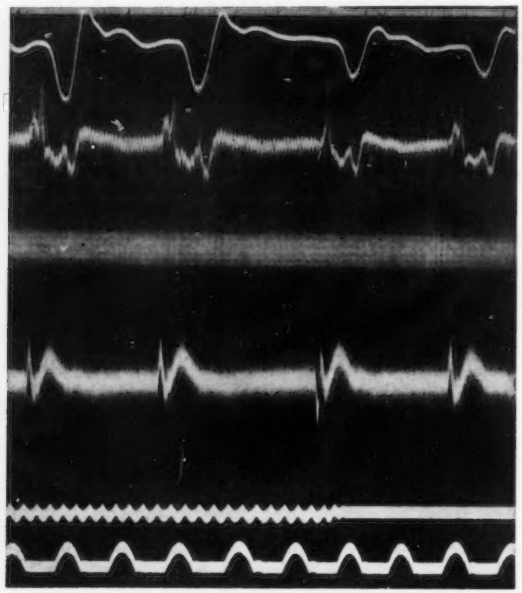

FIGURE 1. Simultaneous comparisons of the sinus node with the auriculo-ventricular node (upper curve) and of the upper part of the sinus node with the lower (lower curve). The third cycle just at the end of vagal stimulation shows a beat arising in the lower part of the sinus node as indicated by the reversal in direction of the auricular wave in the lower galvanometer record.

In all figures the uppermost curve is from a tambour showing the mechanical contraction of the auricle, the second curve is from the string of the upper galvanometer, the third curve is from the string of the lower or second galvanometer, the fourth curve is from a signal showing the duration of vagal stimulation or cooling, and the fifth curve is from a clock marking fifth seconds.

region as shown by the upper curve, the point of initial activity, as shown by the reversal in the lower curve, had shifted from the upper to the lower part of the sinus node. In this case the condition did not persist, for the fourth cycle shows a return of the curves to their original forms.

In Fig. 2 is reproduced a portion of a record showing the

return to normal after a left vagal stimulation which had lasted three seconds. A reversal of the lower curves was produced which consisted of 14 beats and outlasted the stimulation two and one half seconds. The last two cycles of this series are the first two showing in the figure. The return of the pacemaker to the upper

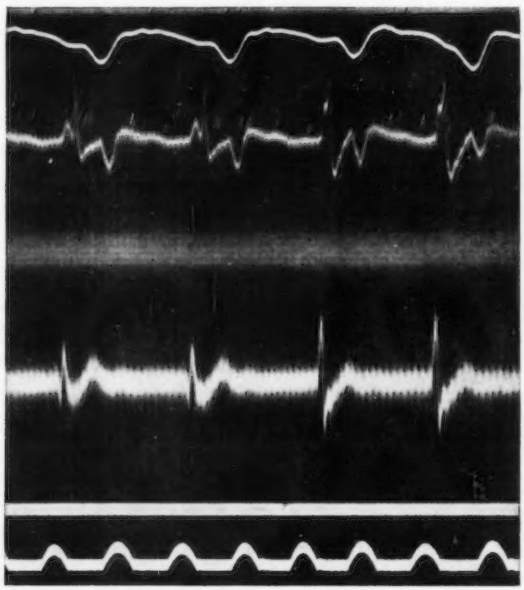

FIGURE 2. Comparisons the same as in Fig. 1. Impulse formation was shifted to the lower part of the sinus by vagal stimulation. The reversal of the auricular wave in the third cycle of the lower galvanometer shows the return to normal.

part of the sinus node is made evident by the third and fourth cycles of the lower curve beginning with an upstroke. It is such examples as these which have led us to conclude that vagal stimulation may depress only a part of the sinus node, allowing another part to take up the pacemaking function.

The ultimate automaticity of a part is determined by its rate of discharge. If according to the hypothesis of vagal action which will be presented later in this paper, a region of high automaticity, the upper part of the sinus node, has been depressed by vagal stimulation, and a region of less automaticity, the lower part of the

sinus node, has taken on the pacemaking function, then the heart rate should be slower. This idea is amply borne out by our records. The second cycle in Fig. 1 is about .08 sec. longer than the average. In Fig. 2 the cycles showing reversal of the lower curve are .03 sec. longer than the normal.

Although most of the cycles occurring with the pacemaker in the lower part of the sinus node have shown shortened As-Vs intervals as may be seen in the third cycle of Fig. 1, we have not felt like drawing conclusions from such results since the As-Vs interval may be greatly modified by the dromotropic influence of the vagus. Such data we believe will be much more valuable from experiments in which the sinus node is depressed in some way, as by localized cooling, which would not produce a widespread influence on conduction.

In several records we have observed during vagal stimulation a splitting of the auricular wave in the sinus node — auriculo-ventricular node lead. Examples of this may be seen in Figs. 1 and 2, the condition disappearing in the latter record as vagal influence ceased and the pacemaker returned to the upper part of the sinus node. We have previously shown [1] that a sino-auricular interval exists amounting to about .025 sec. In these curves it seems that the sino-auricular conduction was so depressed by vagal action that the contraction of sinus as well as auricle produced its effect on the galvanometer. This occurrence we have noted in previous experiments and have discussed its possible significance in a former paper.[1] The lower record does not show the two waves since here the two electrodes were each on sinus tissue and the contraction of the auricle produced little or no effect.

In our experiments the left vagus was much more effective in shifting the pacemaker to the lower part of the sinus node than the right. In only two cases did we secure results from the right vagus. This at first seems somewhat contradictory to the recent work which is in favor of a relative homo-lateral distribution of the vagi, but on closer analysis our results rather support such conclusions. Removal of the pacemaker from one part of the sinus node to another can only be brought about by weak vagal stimulation. This is evident from the absence of extra systoles,

[1] Eyster and Meek: Archives of internal medicine, 1913, xi, p. 204.

reversed cycles and block in all our successful records. This proper degree of stimulation may have been easiest obtained with the left vagus for the very reason that it sends fewer fibers to the sinus node. Our stimulations of the right vagus were usually strong enough to produce extra systoles and other irregularities and consequently fewer cycles with the pacemaker removed merely to the lower part of the sinus mode.

2. *Cooling the Upper Part of the Sinus Node with Ice and Ethyl Chloride.* — It seemed to us that if it was the depressant action of the vagus on the upper part of the sinus node which allowed the lower part of the node to express its automaticity by assuming the pacemaking function, then any procedure which would depress the upper part of the node ought to give similar results. Experiments were therefore planned in which the upper part of the sulcus terminalis was cooled with ice or ethyl chloride sprays. Seven such experiments were carried out in each of which long series of reversals were obtained indicating that the pacemaker had shifted to a lower part of the sinus node.

In Figs. 3 and 4 may be seen the removal of the pacemaker to the lower part of the sinus node and its return as portrayed by the electrical curves. A pencil of ice was applied to the upper part of the sulcus terminalis two seconds before the beginning of Fig. 3. The sixth cycle of the figure shows a reversal of the auricular wave in the lower curve. Between Fig. 3 and Fig. 4 a space amounting to four and one half seconds of the record and including 10 cycles with reversed auricular waves has been omitted. In the fourth cycle of Fig. 4 the auricular wave of the lower curve begins with an upward stroke, showing the return of initial activity to the upper part of the sinus node.

This shifting in the point of negativity we have secured repeatedly in each of the seven experiments by cooling either with ice or ethyl chloride. That the response was due to some specific effect on the upper part of the node was proved in each experiment by cooling the lower part of the sulcus terminalis. This procedure was invariably ineffective. On freezing the entire sinus node with ethyl chloride auriculo-ventricular rhythm appeared.

In these experiments as in the previous ones concerned with vagal stimulation, the descent of the pacemaker to a lower part of

the sinus node was always marked by a lengthened cardiac cycle. This is best seen in Fig. 4 where the shortening of the cycles as the pacemaker returns is easily noted. The length of cycle during the time in which the auricular wave was reversed equalled .458 sec. This shortened down to .411 sec. as the pacemaker migrated upward.

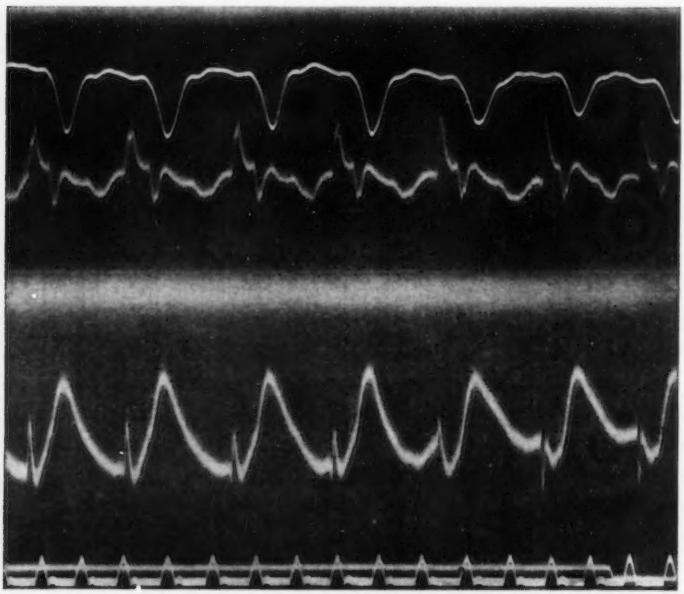

FIGURE 3. Effect of cooling the upper part of the sinus node for three seconds with a pencil of ice. The reduction and final reversal of the auricular wave in the lower galvanometer curve indicates the assumption of the pacemaking function by the lower part of the sinus node.

Another point of great interest was the shortening of the As-Vs interval which occurred as the pacemaker moved downward. This may be most clearly seen in Fig. 3. The As-Vs interval of the first two cycles equals .102 sec. while in the last cycle of the figure the interval has become reduced to .091 sec. This reduction though slight has been constant. Its significance will be discussed in the next section.

3. *Injection of KCl.* — On the basis of Howell's[1] theory that vagal inhibition is due to a liberation of K ions in the automatic tissues it seemed that a migration of the pacemaker from the upper part of the sinus node might be expected if an amount of KCl just sufficient to depress this part could be injected into the blood stream. This was accordingly tried in three experiments

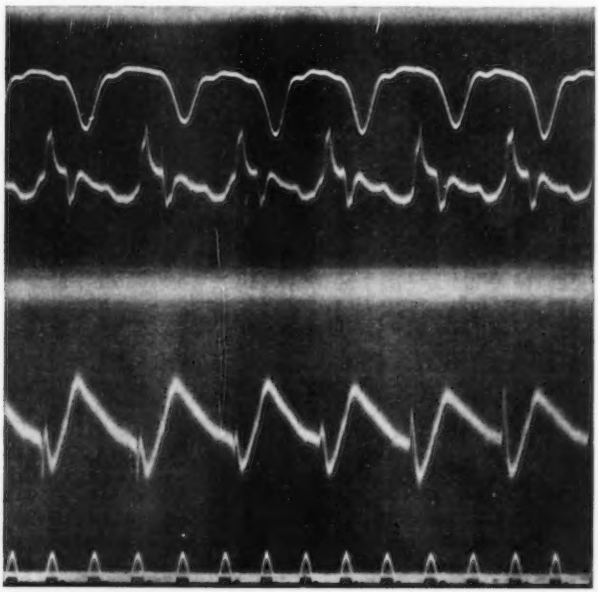

FIGURE 4. This is a continuation of Fig. 3 taken four and one half seconds later. As the effect of the cooling wears off the auricular wave of the lower galvanometer reverses, indicating the resumption of the pacemaking function by the head of the sinus node. Throughout all records the direction of the auricular wave in the upper galvanometer has remained unchanged, showing that at all times the seat of primary activity was in the sinus region.

and in a fourth the sulcus terminalis was painted with 5 and 10 per cent KCl solution.

In two of the experiments following injections of KCl there was a reversal of the lower curve showing that the pacemaker had left the upper part of the sinus node. In the first of these cases the condition was brought on by an intravenous injection of 6 c.c.

[1] HOWELL and DUKE: This journal, 1908, xxi, p. 51.

of a 5 per cent KCl solution in 1½ minutes. This had, however, been preceded shortly before by an injection of 4 c.c. In this experiment the tracings did not return to normal and investigation showed that a coronary sinus rhythm had been produced. This might be readily explained on the basis that the dose of KCl was large enough to paralyze the entire sinus node. In another experiment following an injection of 10 c.c. of a 5 per cent KCl solution in 1½ minutes there was a shifting of the pacemaker from the upper to the lower part of the sinus node as shown by reversal of the lower curve and the absence of any change in the upper. The curves later returned to normal.

Although these experiments are few in number they show that in KCl we have another means of depressing the upper part of the sinus node with the assumption of impulse formation in some lower part. This elective depressant action would seem to lend support to Howell's theory of vagus inhibition.

Discussion

Most of the recent physiological work has emphasized the part played by the specialized tissue of the heart in the initiation and conduction of excitation. Our own work has shown that with the heart in situ beats arising outside of the specialized tissue are extremely infrequent, if they occur at all. By electrical methods we have found in agreement with Ganter and Zahn [1] and Zahn [2] that if the sinus node as a whole is destroyed, depressed or isolated some lower part of the specialized tissue at once takes on the pacemaking function. Strong vagal stimulation was found to be an effective means of depressing the entire sinus node and producing auriculo-ventricular beats. In the present paper we have shown that with weak vagal stimulation or other means of moderate depression such as local cold, the seat of impulse formation may migrate from the upper to the lower part of the sinus node.

Following the principle long ago laid down by Gaskell and Engelmann and so often insisted on by Hering, that the most

[1] Ganter and Zahn: Archiv für die gesammte Physiologie, 1912, cxlv, p. 335.

[2] Zahn: Archiv für die gesammte Physiologie, 1913, cli, p. 247.

automatic part of the heart is the pacemaker at that given moment, our experiments detailed above have led us to the following conception of the action of the specialized tissue and the vagal mechanism of the heart. The specialized tissue of the heart exhibits from above downward progressively diminishing degrees of automaticity. When the dominant rhythm of the heart arises from the highest parts of this system the rate is maximal, other conditions remaining constant, and when it arises from the lowest parts the rate is minimal. Intermediate rates result from some part between these assuming the rôle of pacemaker. Each part of the specialized tissue has of course its own maximum and minimum rate, the exact rate at which it functions at any time depending on nervous influences, temperature and other factors affecting it. It is this maximum which is highest for the upper part of the specialized tissue, that is the sinus node, and which decreases progressively downward.

Those fibers of the vagus that influence the rate of the heart, the chronotropic fibers, are distributed to the specialized tissue comprising the sino-auricular and auriculo-ventricular nodes and their branches. The specific function of the chronotropic fibers of the vagus is to depress automaticity in the specialized tissue. The mass of innervation, that is to say the number of fibers distributed to any region, is an important factor in determining the amount of effect produced on this region when the vagus trunk is stimulated. With weak stimulation of the vagus only those regions would be affected which receive a proportionately large number of fibers. With stronger stimulation the effect might spread to other regions of specialized tissue receiving a less profuse chronotropic innervation. The greater number of vagus chronotropic fibers are distributed to the most automatic part of the sino-auricular node, that part which normally acts as pacemaker for the whole heart. Other parts of the sinus node, with a smaller degree of inherent rhythmicity, receive relatively fewer chronotropic fibers. The auriculo-ventricular node and its connections, representing that part of the specialized system which has a relatively lower degree of automaticity, receives a still smaller number of chronotropic vagus fibers.

Light vagal stimulation, as in stimulation of the vagus trunk

with a weak electrical current, will affect to an appreciable degree only that part of the sino-auricular node which possesses the highest degree of automaticity. The automaticity of this part will be depressed until it is lower than that of some other portion of the node and the latter will at once assume the control of the heart rhythm, or in other words, become the pacemaker. The net result is a slowing of the whole heart.

To give a concrete example one may suppose that there are two points within the sino-auricular node, *A* and *B*, which have different degrees of automaticity, such that *A* is able to excite excitations at the rate of say 100 to 70 beats per minute, while *B* can initiate impulses as a result of its inherent automaticity only at rates between say 80 and 50 per minute. Unless there is still a third region which has a higher rate of discharge, *A* will dominate all other regions and will act as pacemaker for the whole heart at a rate somewhere between 100 and 80. If now the vagus is stimulated, and if this nerve due to more profuse distribution of its fibers to *A* has a greater influence on this region than on *B*, then the automaticity of *A* may be so reduced that its power to discharge impulses will fall below that of *B*, say to 70 or 75 per minute, *B* not being markedly depressed since it has a less profuse vagal innervation, will now be the most automatic part of the heart and will at once assume the rôle of pacemaker for the whole heart. Change in rate is due first to depression of *A* and second to a change in the seat of impulse formation from a point of higher to lower automaticity.

Still stronger stimulation of the vagus may now depress the power of *B* and the pacemaking function will then be assumed by a third region of still lower automaticity. In this way it may be understood how a stronger and stronger stimulus may cause a progressive reduction in heart rate accompanied by a migration downwards of the pacemaker. If finally the stimulation of the vagus becomes sufficiently strong so that even the lower parts of the specialized tissue with their poor innervation are depressed, all impulse formation may cease and we have for a time a complete vagal inhibition of the heart.

We are aware that there have been intimations of some such conception of the automatic tissues of the heart and of vagal action

as that presented above, but we do not believe it has been previously presented in a complete form and certainly it has never been supported by the physiological evidence now at hand. This theory of the specialized tissues in the heart and of vagal action has support in and has been deduced from the following experimental facts.

1. As shown in the present paper slight degrees of vagal stimulation may cause the pacemaker to migrate from the upper to the lower part of the sinus node. This change is always accompanied by a slight slowing in rate.

2. Other depressing agents such as ice and ethyl chloride when applied to the upper part of the node cause the seat of impulse formation to remove to lower parts of the node.

3. If the sinus node be subjected to extremes of vagal stimulation or cooling with ice and ethyl chloride, or if the node be injured, destroyed, or isolated by crushing, cutting or the application of drugs, the pacemaker of the heart migrates temporarily or permanently to lower parts of the specialized tissue, usually the auriculo-ventricular node.

4. Flack [1] has shown that there is a profuse supply of chronotropic fibers to the sino-auricular node, greater than to other parts of the heart.

5. In auriculo-ventricular rhythm, in which the pacemaker resides in the auriculo-ventricular node, the chronotropic action of the vagus is very much reduced.

The shortening of the As-Vs interval observed in our experiments when the lower part of the sinus node became pacemaker seems to us of considerable interest. The gradual shortening of this interval, sometimes seen as auriculo-ventricular rhythm appears or disappears, has always been a difficult thing to understand. We have recently ventured to suggest that such variations of the As-Vs interval were in large measure associated with a shifting of the physical location of the pacemaker. The data now at hand seems to substantiate that view.

We have previously shown [2] that conduction from the sinus

[1] Flack: Journal of physiology, 1910–11, xli, p. 64.

[2] Eyster and Meek: Heart, 1914, v, p. 119.

node to the auriculo-ventricular node cannot be by way of the auricle. The path to the auricle is probably a diffuse one directly across the sulcus terminalis while the path to the auriculo-ventricular node is a linear one, just how well circumscribed we are not able at present to say. As the seat of impulse formation passes downward the time of access to the ventricle shortens and that to the auricle remains the same or lengthens. Since it is now known that the lower part of the sinus node and the auricular portion of the auriculo-ventricular node may act as pacemaker, the gradual shortening of the As-Vs interval may well be explained in large part at least by the migration of the pacemaker through these regions.

On the hypothesis that the specialized tissue of the heart is the seat of all automatism and conduction, then any point to which the pacemaker is forced should lie in this system. The migration of the seat of impulse formation from the head of the sinus node first to a lower part of the node thus becomes very suggestive. If there is a definite path between the sinus node and the auriculo-ventricular node, then this new seat of impulse formation should be in it. If there is a special path from the higher auricular parts to the lower, it seems that it must pass through the lower part of the sulcus terminalis. If the connection between the sinus node and the auriculo-ventricular node is diffuse, then the migration of the pacemaker to the lower part of the sinus node has no interest so far as a circumscribed path of conduction is concerned.

Summary

With the aid of two string galvanometers, one comparing the upper with the lower part of the sinus node and the other comparing the sinus node with the auriculo-ventricular node, it has been shown that by means of vagal stimulation, cooling of the upper part of the sinus node or injection of potassium chloride, the point of initial negativity may be made to shift from the upper to the lower part of the sinus node. This has been interpreted as showing that during these procedures the pacemaker of the dog's heart may move from the upper to the lower part of the sinus node.

During the time that the seat of impulse formation resides in a lower part of the sinus node there is a lengthening of the cardiac cycle and a shortening of the As-Vs interval.

On the basis of work presented in this paper and others of the series, a theory has been presented which correlates our experimental results on the automatic and vagal mechanisms of the vertebrate heart. It is believed that the specialized tissues of the heart exhibit from above downward progressively diminishing degrees of automaticity. Vagal chronotropic innervation of the specialized tissue also diminishes from above downwards. The most automatic portion of the specialized tissue acts as pacemaker for the heart. The function of the chronotropic fibers is to depress this automaticity. When the automaticity of the pacemaker is reduced below that of a lower part the latter assumes dominance and becomes pacemaker. In this way the vagus, if the stimuli are properly graded, may cause the pacemaker to descend from the upper part of the sinus node where it resides normally, to the lower part of the sinus node, and finally even to the auriculo-ventricular node.

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REACTIONS OF EARTHWORMS TO HYDROXYL IONS

By A. T. SHOHL

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I. Introduction

Parker and Metcalf ('06) demonstrated the effect of salt solutions, and Hurwitz ('10) of acids, on earthworms. At Prof. G. H. Parker's suggestion I attempted to determine the effect of hydroxides on earthworms.

The worms were immersed in solutions of hydroxides. They withdrew from it when possible. This phenomenon can be regarded as a physiological reaction to a stimulus; there are nerves in the epidermis whose stimulation results in the contraction of muscles. The animal can also be considered as one whose cellular tissue is composed of proteins in colloidal solution. The environment can be regarded as solutions which are dissociated into positive basic ions and negative hydroxyl ions. It is this latter point of view that I adopt for the purposes of the present work. The problem then becomes the mechanism of protoplasmic stimulation by electrolytes.

Under the conditions of the experiment the worms withdrew from all solutions. The different ions effect the specific nerve endings, quantitatively not qualitatively. The hydroxides of sodium and potassium were compared as to their relative intensity as a source of stimulation; the hydroxyl ions and basic ions were compared as to which was the greater factor in producing the effect. Several of the factors involved were investigated, — namely: a non-stimulating solution in which to keep the worms, the effect of distilled water, and that of temperature. In order to improve the conditions of the experiment a new method was devised for submitting the worms to the solution.

The results obtained in the investigation were subjected to an analysis of the physicochemical factors involved. The conclusion derived is that stimulation is dependent on the specific nature of the ion, probably through the electrical action it produces.

II. Method

The method first used was that described by Parker and Metcalf ('06, p. 56). The worms tested in these experiments were Allolobophora foetida. They were obtained from a large manure heap in Cambridge where they could be found all through the winter. At first they were collected fresh every few days. Later this was found unnecessary, for they could be kept for an indefinite time in the laboratory in jars containing manure.

A worm was prepared for the test by being rinsed in a little tap water to remove the dirt. Through the posterior region a silk thread was passed. The worm was then placed in a small, wide-mouthed jar which contained filter paper and a little water. This was so inclined that the worm could crawl in or out of the water. When ready for use the worm was removed from the jar by means of the thread, care being used not to let it touch the sides. It was suspended on the end of a lever arm like a bucket in a well. When it had become extended, it was lowered into the solution to be tested, as far as the anterior edge of the clitellum. As it touched the solution a stop-watch was started and when it withdrew from the solution the watch was stopped. The worm was then taken off, dipped in tap water, and returned to its jar. The time of immersion was recorded and called the reaction-time. The solutions were taken so that each worm was used first in some one solution and then was dipped in every other solution before it was again exposed to the first solution. The object of this procedure was to prevent the worm from being exposed exclusively to one solution, and hence to permit direct comparisons. Every worm was first in some one solution. This was meant to eradicate the possibility of the first immersion influencing the subsequent trials. Averages of eight or more readings were taken to obviate the error of individual results.

The solutions used were made up from the purest obtainable

sodium and potassium hydroxide sticks (Merck). The stock solutions, from which the others were made as needed, were in semi-normal concentrations. These were titrated against semi-normal oxalic acid with phenolphthalein and methyl-orange as indicators. The former registers only the hydroxyl ions; the latter, total alkalinity.

	Phenolphthalein:	Methyl-orange:
NaOH	$.97\frac{m}{2}$	$1.28\frac{m}{2}$
KOH	$.85\frac{m}{2}$	$1.21\frac{m}{2}$

III. Some Factors in the Reaction

Variation in Reaction Time. Even when all the conditions are as nearly uniform as can be obtained, there is a great difference in the reaction of these animals. Even with the same solution on the same day the individual differences may amount to over one hundred per cent in the duration of the reaction. Not only is this true, but as Towle ('04) has found for paramoecium also, animals change in reaction from day to day and with the seasons. The condition of breeding may also be a factor, according to Towle ('04). The size of the worm is of some importance; the larger the worm, the quicker it reacts. In this work, worms of about the same size were therefore selected. These and many other conditions which could not be analyzed must surely affect the reaction.

Number of Reactions. Even when used as a direct comparison between solutions, the factor of fatigue enters. In order to determine for how many trials a single worm could be used, eight worms were subjected to forty trials each. At the end of that time they were beginning to show fatigue. The reaction-time was lengthening. They seemed to be stimulated but pulled themselves out of the solutions with difficulty. Hence, worms should not be used for many reactions; better not above twenty-four, for after this the reaction-time lengthens.

Stimulation by Distilled Water. Although worms can live in water for days, they withdraw rapidly from it if possible (see Table II). So the fact that they can maintain life does not mean that

water is not stimulating, as Towle ('04) and others have said. The following work was suggested by that of G. Bullot ('04). He speaks of the toxicity of pure distilled water and says this is ordinarily due to the dissolved copper salts. He determined, however, that very pure water which he distilled several times, using Jena glass, quartz, and platinum stills, continued to have a toxic effect on Gammarus. The distilled water supply available for my work was kept in copper vats. Some distilled water was redistilled carefully, using Jena glass stills, one containing potassium permanganate and the other sulphuric acid. The water was collected through block tin condensers, received in clean Jena flasks and used the same day. It was compared with ordinary tap water and ordinary distilled water (see Table I). The tap water was less stimulating than the distilled water and slightly more stimulating than the redistilled water.

TABLE I

AVERAGE REACTION-TIME IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO TAP, DISTILLED, AND REDISTILLED WATER. DEC. 17

Number of the worm	Water		
	Distilled	Tap	Redistilled
I	9.1	8.4	12.7
II	8.7	12.0	13.2
III	11.6	14.3	15.1
IV	12.8	16.6	15.0
Average	10.5	12.8	14.0

Physiological Solution. Not only is distilled water more stimulating than tap water, but the latter is more effective than dilute hydroxide solutions (see Table II, page 388).

Parker and Metcalf ('06) found that for salts dilute solutions were less stimulating than water. An attempt was made to find a solution which was physiological for worms. Parker and Metcalf ('06, p. 67) have shown that worms which live in manure and in

TABLE II

AVERAGE REACTION-TIME IN SECONDS FOR EIGHT TRIALS EACH OF A SERIES OF EARTHWORMS TO A SOLUTION OF POTASSIUM HYDROXIDE AND WATER. FEB. 26

Number of the worm	H_2O (Before KOH)	KOH $\frac{m}{250}$	KOH $\frac{m}{350}$	KOH $\frac{m}{400}$	H_2O (After KOH)
I	12.2	5.5	12.0	19.8	12.4
II	11.7	3.9	24.4	51.5	13.2
III	10.8	7.2	27.0	15.6	20.0
IV	14.4	6.0	14.0	22.8	10.6
Average	12.3	5.6	19.3	27.4	14.0

garden soil differ, a fact which they attribute to the chemical environment. If this were so, a solution of the inorganic salts in approximately the same concentration as in manure should not be stimulating.

Professor Parker kindly obtained for me through the New Haven Agricultural Department the following analysis of manure (Wolff):

K	.53	NO_3	.58	H_2O	71.3%
Na	.10	Cl	.04	Organic	25.4%
Ca	.21	SO_4	.07	Other	3.3%
Mg	.14	PO_4	.28		
$Fe(OH)_3$	.11	Silica	1.27		
Al_2O_3					
	1.09		2.24		100.0%

On this basis, molal solutions of the nitrates of potassium, sodium, calcium, and magnesium were made. The solution employed contained the following amount of salts:

KNO_3	N	98.59 c.c.
$NaNO_3$	N	31.89 c.c.
$Ca(NO_3)_2$	2N	38.89 c.c.
$Mg(NO_3)_2$	2N	42.73 c.c.
H_2O		787.90 c.c.
		1000.00 c.c.

This solution is practically decinormal as regards the potassium, but almost three times as concentrated (.294N) in respect to the nitrates. This was further diluted and tried in different strengths. The longest reaction-time, over three minutes in some cases and averaging one and a half minutes, occurred in 1/50 concentration of the solution. In both greater and less concentrated solutions the reaction-time was shorter. In the later experiments ten cubic centimeters of this solution S/50 was kept in the jars; this did not affect the reactions of the worms to the hydroxide, as was found by controls in water, but did give more even results and seemed to keep the worms in better condition. These solutions are obviously only a rough approximation to a physiological solution. In S/50 the concentration is:

$$K = \frac{m}{500}, Na = \frac{m}{1500}, \quad Ca = \frac{m}{1250}, \quad Mg = \frac{m}{1250}, NO_3 = \frac{m}{175}.$$

No attempt was made in this work to vary the concentration of the kations nor to introduce other anions. This could be done on theoretical consideration and would probably give better results. However, this solution is interesting in that it is different from the Ringer solutions commonly used. The behavior to it seems to bear out the evidence just quoted, that reaction to neutral salts is dependent on the chemical environment. This solution is certainly very different from the concentration of salts in sea water.

Temperature. It is a striking fact that the literature makes so little reference to the effect of temperature on worms. Towle ('04) mentions casually that cold retards the action. It is well known that the speed of chemical reactions is increased with the rise of temperature. This has been worked out in minute detail for the effect on the periods of latency, contraction and relaxation of both smooth and striated muscle. It has been shown that

temperature affects both the irritability and velocity of conduction of nerves. In general, the rule of Van't Hoff may be applied; i.e., that for every ten degrees rise in temperature, the velocity of reaction increases from one to two fold for physical processes and from two to three fold for chemical reactions.

Experiments in which the temperatures were varied indicated a marked effect which was constant and important. The results are shown in Table III and plotted in Figure 1.

TABLE III

AVERAGE REACTION-TIME IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO SODIUM HYDROXIDE $\frac{m}{500}$ AT VARYING TEMPERATURES

Number of the worm	17° C.	21° C.	24° C.	27.5° C.
I	30.2	42.0	22.5	19.2
II	23.9	8.9	12.0	9.7
III	16.7	10.6	13.7	6.6
IV	25.1	20.8	12.9	7.8
Average	23.9	20.6	15.3	10.8

Small variations in the temperature made a marked difference in the reaction-time, which shortens as the temperature increases; i.e., the higher the temperature, the more the stimulation. With a rise of ten degrees, the velocity of the reaction is a little more than doubled. This would place the response in the category of chemical reactions.

In the later work a thermostat was employed. A large basin of water was heated with an electric light. The temperature was regulated to within half a degree by a mercury bulb which dipped into the water and made and broke the current in the light. The water was kept at a uniform temperature throughout by a glass suction stirrer run by a small motor. The whole was contained in a double-walled, padded chamber. This kept the air at about the same temperature as the water. The apparatus was placed in the

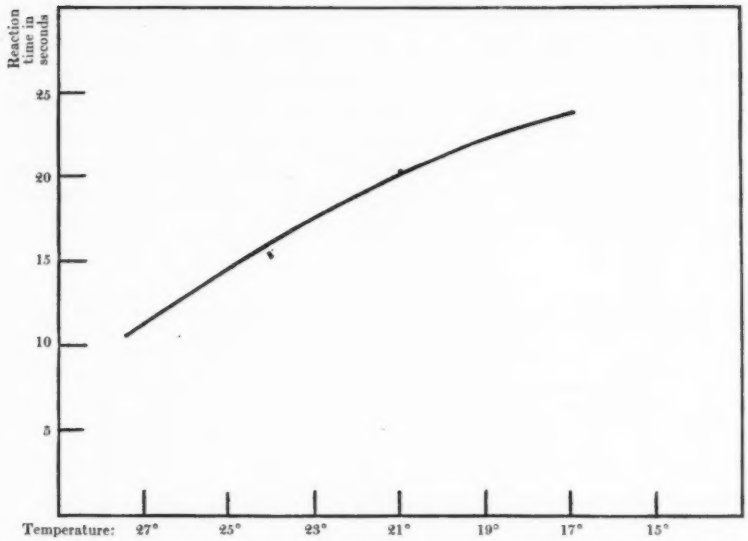

FIGURE 1. Effect of Temperature (plotted from Table VII). Reaction time in seconds.

water so that the solutions were kept at constant temperature. All subsequent work was done at 25 degrees C.

IV. Effect of Hydroxides on Reaction-time

The object of this part of the work was to determine the effect of varying concentrations of sodium and potassium hydroxides on the earthworms and then to compare their effects at the same dilution. First, semi-normal sodium hydroxide was tried. The first worms showed signs of intense irritation; they threw out yellow, malodorous secretions all over their bodies and died immediately. They withdrew from a twentieth normal solution the way a person would from fire. The limits of the reaction lie approximately between $\frac{m}{200}$ and $\frac{m}{800}$. At the latter dilution they remained in the solution a considerable time; below the former dilutions they withdrew so rapidly that no accurate reaction-time could be taken.

The effect of NaOH is shown in Table IV, graphically plotted in Figure 2. The results show that with varying concentration of sodium hydroxide employed, the stimulus depends directly on the amount of the base present. The effect of KOH is shown in Table V, and in Figure 3. The results with potassium hydroxide are practically identical with those found for sodium hydroxide. These reactions were repeated a number of times and the cases selected are typical ones. So the conclusion can be drawn that for these

TABLE IV

AVERAGE REACTION-TIMES IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO VARYING CONCENTRATIONS OF SODIUM HYDROXIDE.. FEB. 18, 25° C.

Number of the worm	$\frac{m}{350}$	$\frac{m}{400}$	$\frac{m}{450}$	$\frac{m}{500}$
I	6.5	22.5	22.4	27.2
II	2.0	7.4	27.5	34.9
III	1.0	10.0	16.3	13.3
IV	0.8	13.5	16.1	20.0
Average	2.5	13.3	20.5	23.8

TABLE V

AVERAGE REACTION-TIME IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO VARYING CONCENTRATIONS OF POTASSIUM HYDROXIDE. FEB. 21, 25° C.

Number of the worm	$\frac{m}{350}$	$\frac{m}{400}$	$\frac{m}{450}$	$\frac{m}{500}$
I	7.9	5.8	12.2	10.9
II	—	20.0	23.0	29.8
III	21.5	27.0	24.0	19.0
IV	14.0	27.0	28.0	38.0
Average	14.4	19.9	21.8	24.4

dilutions the speed of the reactions is directly proportional to the concentration of the base, — the stronger the solution, the quicker the worm withdraws.

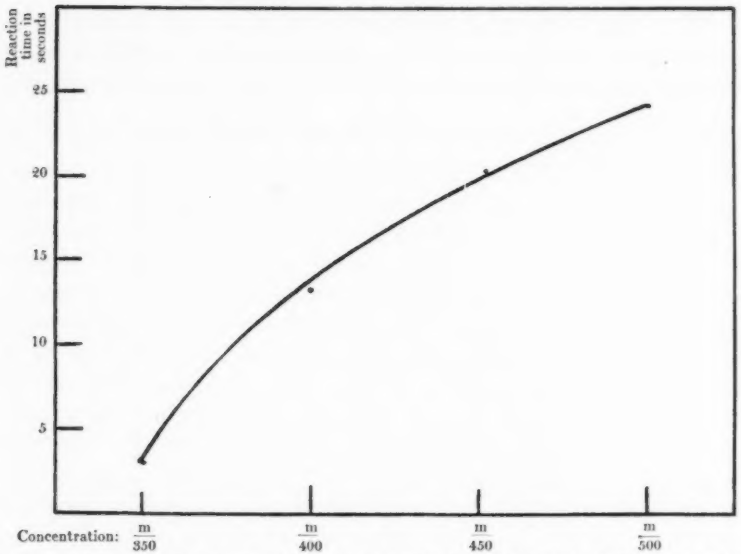

FIGURE 2. Effect of Sodium Hydroxide (plotted from Table IV). Reaction time in seconds.

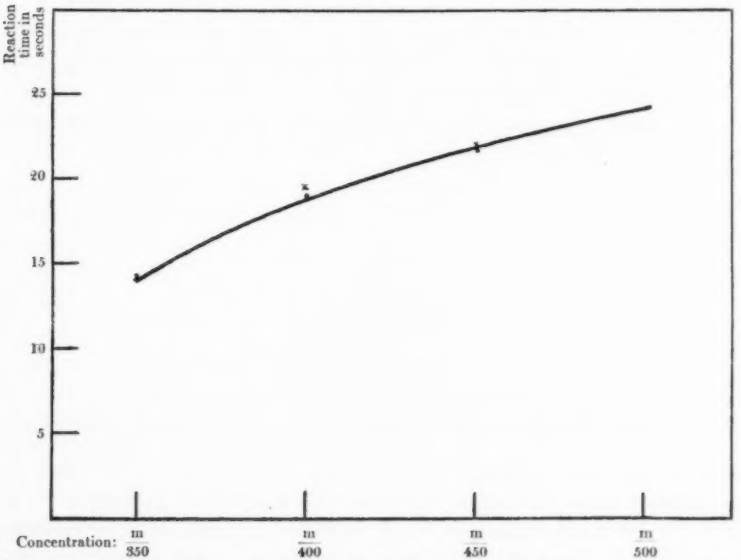

FIGURE 3. Effect of Potassium Hydroxide (plotted from Table V). Reaction time in seconds.

That this is not true for all concentrations has already been pointed out when it was shown that the worms withdrew more quickly from water than from dilute solutions. Beyond a dilution greater than $\frac{m}{1000}$ the reaction-times shorten till the effect of pure water is met. There is a point of dilution at which the hydroxide which is present retards the reaction-time as compared with pure water. This means that there is more than one factor involved.

Since it was known that sodium and potassium hydroxide (within the limits of the dilutions used) were both stimulating in proportion to the amount of base present, it was necessary to have a direct comparison between the two solutions of the same strength to determine their relative effects. The procedure was as in previous cases, except that the different basic solutions were used instead of varying concentrations of the same base. The two solutions were not of quite the same strength, due to differences in the stock solutions, but this difficulty was overcome by interpolating the true value from the curve obtained from plotting the values of different concentrations. (This is justifiable from the uniformity of results.)

The hydroxides of sodium and potassium in the same concentrations are compared in Tables VI and VII. This shows that the reactions are very nearly equal.

The reaction-time is somewhat shorter with sodium than with potassium hydroxide.

$$\frac{NaOH\ \frac{m}{400}}{KOH\ \frac{m}{400}} = 93\%$$

$$\frac{NaOH\ \frac{m}{300}}{KOH\ \frac{m}{300}} = 99\%$$

At $\frac{m}{300}$ the KOH is more dissociated than NaOH and hence should be more stimulating (see page 28) if the stimulation is due only to the hydroxyl ions, but as Parker and Metcalf ('06, p. 58) showed that sodium ions are more stimulating than potassium ions, it may be deduced that the effect was due mainly to the

TABLE VI

REACTION-TIMES IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO SODIUM HYDROXIDE $\frac{m}{400}$ AND POTASSIUM HYDROXIDE $\frac{m}{400}$. MAY 14 AND 16. 25° C.

Number of the worm	NaOH $\frac{m}{400}$	KOH $\frac{m}{450}$	Correction Factor KOH $= \frac{m^1}{400}$
I	25.9	29.1	
II	20.9	29.4	
III	32.2	40.5	
IV	35.3	43.5	
Average	28.5	35.6	30.0

[1] The correction factor is obtained by calculating the relative value for the desired concentration from the curve plotted in Fig. 3.

TABLE VII

REACTION-TIMES IN SECONDS OF THE SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, TO SODIUM HYDROXIDE $\frac{m}{300}$ AND POTASSIUM HYDROXIDE $\frac{m}{300}$. MAY 27 AND JUNE 7. 25° C.

Number of the worm	NaOH $\frac{m}{300}$	KOH $\frac{m}{340}$	Correction Factor KOH $= \frac{m^1}{300}$
I	38.8	55.3	
II	19.0	20.3	
III	27.0	48.1	
IV	43.0	45.5	
Average	31.9	42.3	32.15

[1] The correction factor is obtained by calculating the relative value for the desired concentration from the curve plotted in Fig. 3.

hydroxyl ions. However, since the sodium is slightly more stimulating than the potassium hydroxide, the effect is an additive one in which both kations and anions take part.

V. New Method

The desire to test more clearly the relative effects of the kations, as well as to get a more constant series of readings, led to the devising of a new method, which I have called the fence method. Many other schemes were tried, but the plan finally adapted was to divide a rectangular glass tray (such as is used in photography) into two compartments by a paraffine partition a quarter of an inch wide. A notch about three-quarters of an inch long and reaching to within half an inch of the bottom was cut out of this partition. Such a notched fence may obviously vary in three ways, — first in height, second in length, and third in thickness. Care must be taken that the notch is not cut too deep, or there will not be enough liquid on either side to cover the worms. If it is not deep enough they cannot reach the bottom easily. It was found that the best lining for the bottom was a paraffine coating. By this procedure the solutions on the two sides of the fence do not mix; they do not even wet the walls.

The worms were not threaded as in the former method, but were simply picked up with a moist toothpick. They were so placed that the clitellum rested on the partition fence, the anterior end in one solution and the posterior in the other. Care must be taken to have the source of light at right angles to the long axis of the dish, for the worms are negatively phototropic.

Under such circumstances a worm can do one of several things: it can stay where it was put; it can enter a solution; it can withdraw from it. If it does the last, it can use a swinging motion like that of an elephant's trunk, or a muscular retraction like a collapsing accordion. It may make all of these movements. Time was recorded as in the previous work. In this series of reactions, in all cases, the head was placed in the hydroxide solution.

This method necessitated but little handling of the worms and therefore they were in a more normal state. Their response is a more natural one, for instead of lifting themselves out of a solution, they crawl out. Loeb ('05, pp. 453 and 479) admitted that his method of determining toxicity was faulty, because it depended on a negative factor, — the cessation of irritability. This proce-

dure gives positive results. Further, it gives a direct comparison between any two solutions.

Fresh solutions were made up before trying this method, so as to avoid the use of the correction factor.

Methyl-orange:	Phenolphthalein:
KOH 1.04 $\frac{m}{2}$	1.01 $\frac{m}{2}$
NaOH 1.04 $\frac{m}{2}$	.957 $\frac{m}{2}$

The reactions with this method agree well. Table VIII shows a few records taken at random to show the actual results of individual reactions.

TABLE VIII

INDIVIDUAL REACTION-TIMES IN SECONDS OF EARTHWORMS TO NaOH $\frac{m}{300}$.

Number of trial	Worm I	Worm II
	March 19 19° C.	March 23 15° C.
I	10.0	8.0
II	13.0	8.0
III	12.4	8.0
IV	8.0	8.0
V	5.0	11.0
VI	15.0	6.2
VII	14.0	7.8
Average	11.0	8.0

The Effect of Hydroxides. The work on the comparison of the hydroxides was repeated with this method. The results are more nearly equal than those obtained in the former method. The sodium hydroxide was $\frac{m}{310}$ instead of $\frac{m}{300}$. Hence, this would tend to obscure the difference slightly since the sodium hydroxide, being very slightly weaker, would be less stimulating.

TABLE IX

AVERAGE REACTION-TIMES IN SECONDS OF A SERIES OF EARTHWORMS, FOR EIGHT TRIALS EACH, FOR WITHDRAWING FROM NaOH $\frac{m}{310}$ AND KOH $\frac{m}{300}$ INTO WATER. MAY 3. AND MAY 21. 18° C.

Number of the worm	NaOH $\frac{m}{310}$ | H_2O	KOH $\frac{m}{300}$ | H_2O	NaOH $\frac{m}{310}$ | H_2O
	Before		After
I	6.9	6.4	6.5
II	3.0	2.6	3.0
III	7.7	8.0	8.0
Average	8.0	7.5	8.0

There were no data for making a correction factor as in the former work, for no comparative results on varying concentrations of the same solution were undertaken. The solutions were so nearly equal that this inequality falls within the experimental error. The results are given in Table IX. They show that the reaction-times are very nearly equal, — slightly longer for sodium than potassium (see page 28).

$$\frac{\text{NaOH } \frac{m}{310}}{\text{KOH } \frac{m}{300}} = 106\%$$

Relative Stimulation of Kation and Anion. By the former method it was shown that the effect of the solutions was in the main due to the OH ions and that the Na ions and K ions had a slight but characteristic effect. Loeb ('05, pp. 463 and 476) is of opinion that it makes no difference to what basic ions the hydroxyl ions are attached, since they are to be considered the stimulating factor. The results with all hydroxides are the same and the hydroxyl is poisonous in dilutions when the basic ion is non-toxic. To test this experimentally, a solution of c.p. NaCl $\frac{m}{300}$ was used,

against a solution of NaOH $\frac{m}{300}$. If the solutions depended for their stimulating property on the sodium, they would be equal. In every case the worms withdrew into the solution containing the NaCl; not only that, but faster than into water alone. In other words, the salt solution was less stimulating than water. Hence, the stimulation is due principally to the hydroxyl ion.

VI. Consideration

The experimental evidence in this investigation points to the fact that NaOH and KOH in dilute solutions are stimulating mainly through the hydroxyl ion and are about equally stimulating for Allolobophora foetida. It remains to consider what can be deduced from this and whether the theories suggested by other investigators can be applied to explain the phenomena.

The reaction is more complicated than it seems at first. It is probably mainly the result of direct stimulation of the sensory mechanism in the epithelium. This leads to a motor reflex causing the animal to withdraw from the stimulating solution. There may be some effect due to the fact that the skin acts as an imperfect semi-permeable membrane.

The hydroxides used were sodium and potassium. These bases when in solution dissociate into Na + and K + basic ions, and OH — hydroxyl ions. The degree of ionization is nearly the same for each. The ionization increases with the dilution and is measured by the specific conductivity. This increase is slightly greater for sodium than for potassium, though the latter is actually more dissociated. The percentage dissociated at $\frac{m}{200}$ is 91.5 per cent for NaOH and 95.0 per cent for KOH; at $\frac{m}{500}$ it is 93.0 per cent for NaOH and 95.8 per cent for KOH. Increase in temperature also increases ionization. At this dilution it is only a fraction of a per cent per degree, but slightly larger for NaOH than for KOH. Hence the conditions of the experiment make the ionization very nearly equal. The difference is slightly in favor of greater ionization for KOH, so that in equal concentrations there would be a

slightly larger number of OH ions in the potassium hydroxide than in the sodium hydroxide solution.

The action of the hydroxyl ion must next be considered. The fact that OH is a strong protoplasmic stimulant has been shown by Loeb ('05, p. 480). It can be tasted in solutions of .006 gr. ions per liter (Höber, '11). Kahlenberg ('98) says OH is sapid at $\frac{m}{400}$ while NO_3 must be as concentrated as $\frac{m}{6}$ to be tasted. Alkalies increase the absorption of water under all conditions (Loeb, '05, p. 516). It also acts as a strong lytic for protoplasm (Lillie, '06). The following is a classification of anions from several widely divergent points of view:

Swelling	Br, Cl, CO_3, NO_3, CH_3COO, SO_4, $C_4H_4O_6$, PO_4 (Fischer)
Swelling	SO_4, Cl, NO_3, Br, I, CNS, BrO_3, OH (Lillie)
Antiprecipitating effect ..	SO_4, PO_4, Cl, NO_3, Br, I, CNS (Pauli)
Taste	SO_4, NO_3, Br, I, BrO_3, OH (Kahlenberg)

In all these cases the order is almost identical for swelling, antiprecipitating and tasting. The OH group is the most effective.

There have been many theories as to the way in which these solutions stimulate. Braeuning ('04) said that stimulation in the frog depends on the diffusion coefficient. $KOH \frac{m}{200} = 1.68$ and $NaOH \frac{m}{200} = 1.12$. Our reactions were so similar that this cannot be important.

Loeb ('05, p. 475) maintained that the toxicity is dependent on the migration velocity. K = 64, Na = 23, OH = 173. Hence, $\frac{NaOH}{KOH} = \frac{196}{237}$. This is not borne out by the facts.

The osmotic pressure has often been declared to be the cause of stimulation. Loeb's ('05, p. 470) work showed that muscles swelled in hypotonic solutions and shrank in hypertonic ones for the first hour. This increases more rapidly than it ought in hypotonic solutions and decreases more slowly than it ought in hypertonic ones. However, isosmotic chlorides of Li, K, Rb, Cs, Mg, Ca, Sr and Ba all have the same effect, so that osmotic pressure must have some effect. Overton showed that plasmolysis in plants did not follow osmotic pressure, and Greeley ('04) showed

that ionic effects prevail over the osmotic effects. Loeb ('05, p. 511) also showed that under certain conditions the osmotic law was not true, for he found that even in a 2.8 per cent salt solution, at the end of eighteen hours, a muscle which had decreased in weight at first, afterward weighed more than normal. He showed further that there was a specific ionic effect KCl 0.7 per cent, causing a 45 per cent increase in eighteen hours, and CaCl 0.7 per cent a 20 per cent decrease for the same time, — whereas in LiCl 0.7 per cent the muscle remained constant. This is also evident from a consideration of the reactions obtained by Parker and Metcalf ('06, p. 58) and those in this paper. If the reaction were dependent on osmotic pressure, regardless of the actual length of time, the *longest* reaction in each solution would be in the same concentration. For KCl this occurs at $\frac{m}{50}$; for KOH at $\frac{m}{800}$.

Animal tissue is not an ideal semipermeable membrane. There must be some diffusion. Loeb ('08) has said that in hypertonic solutions, water goes one way and *ions* the other. This would imply, if the action were selective, a condition similar to that of the red blood corpuscles. Lillie ('10, '11) has shown that stimulation causes a change in the permeability of the membranes, so that pigment will diffuse into the solution. This he has called the "sensitizing effect." This runs in the same order as other protoplasmic effects (see page 29), except that he says OH, H and some other non-ionic compounds form irreversible reactions, whereas the anion effects are reversible. If this sensitizing effect were so, the reactions would show a shortening. In fact, we have often noticed that the first reaction is followed by shorter ones for the first few reactions. Then the reactions become constant. Hence the OH cannot destroy the cell membrane in the solutions used. Fischer ('10) has doubted whether there is any membrane, permeable or semipermeable, around the cell. He says it is merely a matter of ions on colloids as influenced by absorption.

Pauli ('07) has shown beautifully the effect of ions on the coagulations of protein, and on the electric charge. Anions are anti-coagulators and electronegative. They act as antitoxins to the kations. Greeley ('04), Lillie ('11a, '11b), Mathews ('04a, '04b, '05), and Sutherland ('06) have applied these principles to

biological problems with excellent results. They conclude that stimulation is dependent on the action current produced by electric charges of the sum of the acting ions. The present work is in agreement with such conclusions.

In this paper an attempt has been made to analyze only one of the factors involved; that is, the cause of the specific effect of the electrolytes as a stimulant. At least two other aspects of the problem present themselves: first, why should certain solutions be non-stimulating? and, second, why should water be more stimulating than dilute solutions containing irritating ions? As far as the electrolytes are concerned there seems to be no doubt that in solutions of hydroxides the effect of the basic ion is of relatively little importance and the solutions are stimulating approximately in proportion to their hydroxyl ion concentration.

VII. Summary

1. Individual dung worms vary in their reaction-time.

2. The average reaction-time varies on different days.

3. Distilled water is more stimulating than redistilled water or tap water.

4. Water is more stimulating than dilute solutions.

5. Worms can be well handled in a "physiological dung solution."

6. Temperature is an important factor; the higher the temperature the faster the reaction.

7. The "fence method" gave more accurate results than the suspension method.

8. Hydroxides are stimulating in proportion to their hydroxyl ions.

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CARBON DIOXIDE PRODUCTION FROM THE NERVE FIBER IN A HYDROGEN ATMOSPHERE

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Introduction

AMONG certain facts which are difficult to reconcile with the physical theory of nerve conduction, the relation of oxygen to the state of excitability is the most important one. In spite of many contrary findings, the facts indicating a dependence of irritability on oxygen in general are now accumulating. Critical studies made by Verworn and his school not only support the idea that irritability is primarily a chemical phenomenon, but also clearly point out some of the fundamental errors which were involved in the earlier work, and were responsible for the present discrepancy.[1]

Under the direction of Professor A. P. Mathews, Tashiro has demonstrated not only that all living nerves give off CO_2, but also that their metabolism increases on stimulation.[2] To demonstrate a further relation between the state of irritability and the metabolic activity of the nerve, we have already reported the facts that nerves stimulated by very weak concentrations of an anaesthetic produce more CO_2 than normally, but when anaesthetized by a higher concentration, which produces a reversible loss of irritability, their CO_2 production is greatly depressed.[3] Whether the different rate of the nerve metabolism is the cause or effect of the change in the state of excitability, it is certain that an

[1] Verworn: Irritability, Yale University Press, 1913, pp. 102, 182.

[2] Tashiro: This journal, 1913, xxxii, p. 107.

[3] *Ibid.*, 1914 xxxiii, p. xxxviii.

active metabolism in the nerve fiber is a fact and the incorrect assumption of an absence of metabolism can no longer be used by the physical school to support their argument.

It is known from the investigations of Verworn and his colleagues that the irritability of nerves is dependent on oxygen; since it has been shown by Tashiro that a very close relation exists between irritability and the power of increasing CO_2 on stimulation, it was very important to determine whether lack of oxygen, which thus lowers irritability, would inhibit or diminish the CO_2 production on stimulation, and whether the CO_2 of resting nerves will be diminished in a hydrogen atmosphere.

Methods and Material

Hydrogen was used in place of air, no particular reason being attached to its use in preference to nitrogen except that of convenience. The gas, furnished by Eimer and Amend, was prepared by the electrolysis of distilled water. It was washed through permanganate, distilled water, and alkaline pyrogallate, and collected by displacement in a large carboy, over a 20 per cent caustic solution, so that every trace of CO_2 might be removed. The gas was further tested for freedom from CO_2 in each experiment with the biometer. This carboy of hydrogen was used in place of CO_2 — free air, and with it the apparatus was filled in exactly the same manner as described elsewhere.[1]

The claw nerve of the Spider Crab was selected on account of our fairly extensive knowledge of its metabolic rate. The nerve is isolated in the manner already mentioned, quickly weighed, and allowed to respire in the apparatus, usually for ten minutes in this gas, and its CO_2 production is quantitatively estimated. The apparatus used exclusively in these experiments was the biometer (apparatus II), with which amounts of CO_2 as small as .000,0001 gm. can be measured.

CO_2 Production from the Resting Nerve in Hydrogen

It appears from this table that in a medium deficient in oxygen the claw nerve of Spider Crab gives off less CO_2 than in the

[1] Tashiro: *loc. cit.*, p. 139.

TABLE I

CO_2 Production from Resting Claw Nerve of Spider Crab, Libinia Canaliculata, in Hydrogen

Date	Temp. of room	Wt. of nerve	Duration of respiration	Vol. of gas taken from respiratory chamber	Ppt. of $BaCO_3$ after 10 mins.	No. of c.c. which gives no ppt., calculated for 10 mg., 10 mins.	No. of c.c. which gives ppt., calculated for 10 mg., 10 mins.	Original vol. of respiratory chamber
Sept. 2	23.8°	37.0 mg.	10 mins.	1.0 c.c.	–	3.70		15. c.c.
"	23.1°	40.5 "	"	1.0 "	–	4.05		"
"	22.8°	27.0 "	"	2.0 "	+		5.40	"
Sept. 3	23.4°	70.0 "	"	.9 "	+		6.30	"
"	23.5°	31.5 "	"	1.0 "	+		6.30	"
"	23.9°	45.5 "	"	1.2 "	+		5.46	"
"	24.1°	33.5 "	"	1.8 "	–	6.03 [1]		"
Sept. 4	22.8°	49.5 "	"	1.0 "	+		4.95	"
"	23.0°	32.0 "	"	1.5 "	+		4.80	"
"	23.0°	36.0 "	"	1.2 "	+		4.32	"
"	22.0°	34.0 "	"	1.2 "	–	4.08		"
"	21.4°	42.0 "	"	1.1 "	+		4.62	"

maximum error

1.0×10^{-7} gms. $\times$ 15/4.32 = 3.4×10^{-7} gms. ($- 0.2 \times 10^{-7}$ gms.)

at 23°.

In air: 6.7×10^{-7} gms. (15–16°)

7.9×10^{-7} gms. (20.2°)[2]

[1] This nerve has been kept in hydrogen for ten minutes, previous to the experiment.

[2] Taken from results obtained in connection with "Effect of Anaesthetics," now in press.

atmosphere. This may mean one of three things, — CO_2 production is diminished, (1) because of lack of oxygen, or (2) on account of the effect of hydrogen, or by 1 and 2 combined. This important point could be decided by substituting in place of hydrogen other inert gases, such as nitrogen. It is to be regretted that we could not perform these experiments because of the lack of facilities at Woods Hole. A variety of facts, however, lead to the inference that it is not due to the effect of the hydrogen. The inertness of hydrogen, which the physiologist experiences in the case of the nerve and other tissues in general, strongly supports our contention that the lowering of the CO_2 output in hydrogen is not due to the presence of hydrogen, but results from the lack of oxygen.

Just what the CO_2 production in hydrogen represents is a matter for further experiment. Two factors may be responsible; either diffusion of already formed CO_2 fixed in the nerve, or its production as a regular end product of metabolism going on anaerobically. In the case of the muscle, where CO_2 is produced continuously in hydrogen, and often is increased temporarily at the outset, the gas is believed to be formed in part by the decomposition of bicarbonate in the tissue, on account of the formation of lactic acid which begins as soon as oxygen is taken away.

That the CO_2 in our experiment must be the expression of a certain phase of metabolic activity which continues to exist without oxygen even in aerobic tissue is not a matter of reckless speculation. The possible mechanism of metabolic activity in the nerve fiber has been considered by Tashiro [1] somewhat as Vernon has suggested in the case of other tissues. He has shown by the action of drugs the possibility of two more or less independent phases of metabolism, one the formation of an organic peroxide between the tissue and oxygen, and the other the decomposition of this peroxide by peroxidase.

From results recently obtained on stimulation of the respiratory center by "oxygen want," Gasser and Loevenhart came to a somewhat similar conclusion.[2] They divide the oxidative process

[1] *Loc. cit.*, p. 135.

[2] GASSER and LOVENHART: Journal of Pharmacology and Experimental Therapeutics, 1914, v, p. 272.

into two phases; one an "activity process" having to do with functional activity, which does not require free oxygen, the other a "recovery process" which consists of the storing up of potential energy and the removal of products of activity, which requires fixation of oxygen by the cell.

Accordingly we may look upon the CO_2 production in hydrogen as a sort of expression of half of the normal metabolism. This mechanism probably will persist long after functional activity has ceased. For not only in hydrogen, but also in conditions of narcosis we have shown that the nerve nevertheless continues to give off CO_2 although in greatly diminished amounts during loss of excitability.[1]

CO_2 Production from "Stimulated" Nerves in Hydrogen

In addition to many physiological facts brought forth by Verworn and his school, Haberlandt has observed that the sciatic nerve of the Frog, at 14° to 19° C. takes up oxygen, — 33.4 to 41.7 c.cm. per gm. per hour. Furthermore, when this nerve is excited the intake of oxygen is increased.[2] Buijtendijk found a similar fact for the cranial nerve of certain fishes, in which the oxygen intake is increased by electrical stimulation.[3] That oxygen is involved in such stimulation of some nerves, at least, seems to be fairly definitely established. Mathews found that salts either would not stimulate a nerve, or their power of stimulation was greatly reduced, if the nerve remained in the body for a time after death, or if the nerve were brought into the salt solution in an atmosphere of hydrogen.

By the preceding it has been shown that CO_2 production is reduced in the resting nerve in hydrogen, and this reduction,

[1] How long such a process can go on without oxygen must depend on the rate of metabolism and the presence of some mechanism to remove the toxic by-products of such metabolism. We show elsewhere that tissues which can stay active in an oxygen-free atmosphere for any length of time have a comparatively low rate of metabolism, even in the air. See "CO_2 production in ganglionated cord of limulus heart," now in press.

[2] Haberlandt: Archive für Physiologie, 1911, p. 419.

[3] Buijtendijk: Koninklijk Akademie van Wetenschappen, Amsterdam, afd. xix, pp. 615–621.

TABLE II

CO_2 Production of Claw Nerve of Spider Crab, Libinia Canaliculata, when Stimulated in Hydrogen

Date	Temperature of room	Wt. of nerve in mgs.	Duration of respiration	Stimulation	Vol. of gas taken from respiratory chamber	Ppt. of $BaCO_3$ after 10 mins.	No. of c.c. which gives no ppt., calculated for 10 mg., 10 mins.	No. of c.c. which gives ppt., calculated for 10 mg., 10 mins.	Original vol. of respiratory chamber
Sept. 3	24.0°	39.5	10 mins.	Stimulated 10 mins.	1.2 c.c.	+		4.74	15 c.c.
"	23.8°	32.0	"	Stimulated first 5 mins.	1.2 "	–	3.84		"
"	22.7°	36.0	"	Stimulated last 5 mins.	1.3 "	–	4.68		"
"	21.6°	35.0	"	Stimulated 10 mins.	1.2 "	+		4.20	"

1.0×10^{-7} gms. $\times$ 15/4.2 = 3.6×10^{-7} gms. (at 21.6°).

judging from the evidence cited, ought to be accompanied by a diminution of irritability. The next question to be attacked was whether there would be an increase of CO_2 output by a nerve electrically stimulated in hydrogen. For this purpose also the claw nerve of the Spider Crab was chosen. Stimulation was effected by an induction coil in a manner similar to that described in earlier papers, using approximately the same strength of current as in air.

TABLE III

COMPARATIVE RATES OF CO_2 PRODUCTION IN THE NERVE WITH AND WITHOUT OXYGEN

Nerve	Medium	CO_2 from resting nerve, calculated for 10 mg.—10 mins.	CO_2 from stimulated nerve, calculated for 10 mg.—10 mins.
Claw nerve of spider crab	CO_2 free air	6.7×10^{-7} gms. (15–16°)	16×10^{-7} gms. (14–16°)
" " " " "	" " "	7.9×10^{-7} gms. (20.2°)	
" " " " "	CO_2, free Hydrogen	3.4×10^{-7} gms. (23.0°)	3.6×10^{-7} gms. (21.0°)

The results given in Table II bring to light an interesting fact. It appears that there takes place practically no increase of CO_2 when the nerve is "stimulated" in hydrogen with weak induction shocks. A close inspection of the table shows possibly a very slight increase of CO_2, which may be due perhaps to experimental error, or is surely within the limits of error. It is certain that there is no such enormous increase as is observed in the case of the nerve stimulated in air.

These results suggest two possible interpretations, — either the nerve when in hydrogen was not stimulated by the same strength of current as stimulated the same nerve in the air, or the CO_2 production from the resting nerve in hydrogen is so small that stimulation under these conditions involves an increase so slight as not to be detected, and the nerve by continued stimulation is quickly exhausted.

Fröhlich[1] found that when a sciatic nerve of a frog is deprived of atmospheric oxygen its irritability, measured by the threshold of stimulation for muscle contraction, decreases more and more, until after the lapse of some hours the stimulation required is so strong as to approach the region of the "stromschleifengreze." If such is the case, the claw nerve also to which is applied such a weak current may be not in reality stimulated. On the other hand Thörner,[2] taking the action current as an index, found that a nerve continuously stimulated in an atmosphere deficient in oxygen is quickly exhausted. It is remarkable that the action current in nitrogen falls to two-thirds of its original value within the first ten minutes. Fatigue of the nerve by continuous stimulation during the first few minutes of our experiments with hydrogen may be brought about.[3]

Whatever interpretation we take, and as a matter of fact both factors may doubtless enter in here, the fact that there is no decided increase of CO_2 on a weak electrical stimulation in hydrogen points inevitably to the view that oxygen is a primary factor in the excitability of the nerve, as well as in the conductance of the nerve impulse.

Discussion

The evidence set forth here that in the absence of oxygen the claw nerve of the Spider Crab has a far lower CO_2 output than in air, and that the application of a weak current, which stimulated the nerve in air and more than doubled its CO_2 output, fails under these conditions to give any decided increase is further proof for the argument that the primary basis of protoplasmic irritability must be a chemical one, as is contended by Mathews, Verworn and others. So far there exist no decided evidence against this metabolic basis of irritability, except the lack of heat production in the case of stimulated nerves so carefully studied by A. V. Hill.[4] This fact, however, we shall later have opportunity to con-

[1] Quoted from Verworn's Irritability, p. 102; see Zeitschrift f. allgem. Physiologie, 1904, Bd. III.

[2] *Ibid.*, p. 185; see *Ibid.*, 1908, Bd. VIII.

[3] Unfortunately we could not test this point on account of lack of time and accommodation in Woods Hole.

[4] A. V. Hill: Journal of physiology, 1912, xliii, p. 433.

sider. The fact remains that there is perfect parallellism between the state of excitability and the CO_2 output, to which the following experimental facts, brought out by studies on the CO_2 production of nerves under various conditions, bear evidence:

1. All irritable tissues give off CO_2, resting nerves being no exception.

2. When irritable tissue is stimulated, this CO_2 production is increased.

3. When a nerve is treated with weak concentrations of anaesthetics, which are known to stimulate or increase irritability, the CO_2 production is likewise increased.

4. When the nerve is treated with higher concentrations, in which reversible loss of irritability takes place, CO_2 production is diminished.

5. In an oxygen-free medium the claw nerve of the Spider Crab shows an exceedingly low CO_2 output.[1]

6. "Stimulation" by a weak electric current in any oxygen-free medium fails to produce any marked increase in CO_2.

Our results on CO_2 production in hydrogen depend on experiments with the Spider Crab nerve only, and are not of themselves sufficient to permit generalization, but they are at least sufficient to indicate that oxygen is primarily concerned with metabolism in the nerve, and they are in harmony with the view that the real basis of irritability may be a chemical one.

We take great pleasure in acknowledging here our indebtedness to Professor A. P. Mathews, under whose direction a series of investigations regarding gaseous exchange in nerve systems has been undertaken, of which series this paper is one.

[1] Some physiologists have called to my attention the possibility that CO_2 production in an isolated new fiber is not a physiological expression, but rather is due to tissue destruction accompanying death; and the increase of CO_2 on stimulation may be due to an acceleration of the death process. The decrease of CO_2 production in oxygen free medium, together with perfect parallelism between CO_2 production and general state of excitability of normal nerve in situ under the similar conditions, should be sufficient evidences against such an argument. For the general consideration on the nature of survival respiration of a tissue, we refer to FLETCHER works: Journal of physiology, 1898–9, xxiii, p. 10.

THE CONDITIONS DETERMINING THE RATE OF CONDUCTION IN IRRITABLE TISSUES AND ESPECIALLY IN NERVE

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THE state of excitation travels in the form of a wave along the irritable element at a rate which is well known to vary greatly from tissue to tissue and from organism to organism. We find, however, when we examine any particular conducting tissue, that the rate of conduction resembles the other physiological properties of the tissue in having a specific character of its own, and that when temperature and other external conditions are constant its value does not vary widely from a certain definite mean. This rate shows no appreciable correlation with ionic or other diffusion-velocities, nor with the transmission-velocities of mechanical, thermal, or electrical influences. It is, however, greatly influenced by changes of temperature, the temperature-coefficient being approximately that of chemical reaction-velocities, and also by changes in the composition of the medium — being retarded, for instance, by anaesthetics. It appears, therefore, that some chemical process or processes must play an important part in the transmission of excitation from one region of an irritable element to another. Any general theory of the nature of the physiological conduction-process must take into account *all* of the above peculiarities and cannot be based on conditions observed in a single irritable tissue alone. A broadly comparative consideration is most likely to lead to theoretical conceptions that will be valid in all cases. Hitherto the nature and conditions of physiological conduction have been investigated chiefly in nerve; but the problem is a general one and in no sense peculiar to this tissue.

It is clear that in any irritable element that responds as a whole to a local stimulus the process initiated at the point of stimulus must spread over the entire element. The manner in which this spread takes place and the conditions determining its velocity in any special instance are the subjects of discussion in the present paper.

The comparative observations on the rate of conduction are most complete for nerve. There are also numerous measurements for striated and heart muscle and fewer for smooth muscle and other tissues. In nerve the velocity of the impulse is greatest in warm-blooded vertebrates, where it may exceed a hundred meters per second, and slowest in sessile animals like mollusca, where it is often no more than a few centimeters per second. Certain general physiological correlations are highly significant. We find throughout the animal kingdom a direct relation between the quickness of muscular response and the velocity of propagation of impulses along the conducting elements.[1] The biological advantage of this is perhaps not so obvious as would appear at first sight; it would seem that what is needed for quickness of motor response is quickness of muscular contraction rather than quickness of nerve conduction, provided this is adequate; yet it is apparently a general rule that where muscular contraction is rapid nerve conduction is also rapid and *vice versa; i.e.*, in any animal rapidity of response is a characteristic of the whole neuromuscular apparatus and not only of certain separate elements. This general fact indicates that there is some condition common to both conducting and contractile elements which determines at the same time both quickness of response and quickness of conduction.

Rapid response implies brevity of latent period and rapid rise of the excitation-state to its maximum. In nerve the only tangible index of the local nerve-process is the electrical variation; the rate at which this variation rises at any point of the nerve from zero to its maximum is a measure of the velocity of the local nervous disturbance. Now the rate of rise of the action-current shows a close parallelism with the velocity of conduction, as the examples about to be cited will illustrate. That the bioelectric process should show this relation to a process of apparently quite different

[1] CARLSON: American Journal of Physiology, 1904, x, p. 401; 1906, xv, p. 136.

nature, the propagation of the excitation-wave, suggests the existence of a direct interdependence, *i.e.*, that the electrical variation as such is the essential change on which the conduction depends. This view was in fact favored by Du Bois-Reymond, Hermann, Kühne, and other early students of the bioelectric phenomena.[2]

There is, however, the possible alternative that some other underlying process determines both the electrical variation and the transmission of the impulse; the electrical variation would on this view be merely an index of the underlying physiological change and might be of no special importance in itself. Such a view appears more consistent with the obvious fact that the propagation of the excitation-wave is readily blocked by conditions that have no influence on electrical conduction, such as ligaturing or locally narcotizing the nerve. On the other hand, the transmission of the state of excitation from one irritable tissue to another by means of the action-current of the first is an equally familiar phenomenon, occurring with perfect constancy under appropriate conditions, and affording direct proof that this kind of transmission is at least possible. Whether or not it is the normal method of transmission of excitation from point to point along the nerve or muscle-fibre is the question which I propose to discuss in the present paper. The existence of blocking effects of the kind just cited is not necessarily inconsistent with this hypothesis, as I shall attempt to show later;[3] it is quite possible, for instance, that electrical effects at the site of the block may interfere with or compensate those that form the basis of the transmission. I shall first review the facts that indicate a dependence of transmission on the bioelectric variations of the active elements, and shall afterwards consider the possible manner in which this transmission may take place.

First, with regard to the parallelism between the velocity of the electrical variation and the velocity of conduction in various irritable tissues. The following table summarizes a large number of observations made by different investigators under different condi-

[2] Du Bois-Reymond: Gesammelte Abhandlungen zur allgemeinen Muskel- und Nervenphysik, Vol. ii, p. 698; *cf.* p. 733. Hermann: *cf.* Handbuch, Vol. 1, p. 256; Vol. 2, i, p. 193. Kühne: *cf.* Croonian Lecture: Proceedings of the Royal Society, 1888, Vol. xliv, p. 446; Zeitschrift für Biologie, xxiv, p. 383.

[3] See page 440.

TABLE

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
A. Striated Muscle:		
Frog's gastrocnemius ...	2.58–3.84 σ [4] (*ca.* 20°)	
" " ...	1.1–3.5 σ [5] (*ca.* 20°)	*ca.* 3–4 met-.sec.[5]
" sartorius	1.6–3.2 σ [6] (*ca.* 20°)	
" "	4.1–4.2 σ (8°)[7]	*ca.* 1.2 met.-sec. (8°)[7]
" "	2.4–2.9 σ (18°)[7]	*ca.* 1.65 met.-sec. (18°)[7]
" "	13 σ (3°)[8]	1.06 met.-sec. (3°)[8]
" "	5.8 σ (14.8°)[8]	1.65 met.-sec. (14.8°)[8]
" hyoglossus	20 σ (3°)[8]	0.38 met.-sec. (3°)[8]
" "	8.9 σ (14.7°)[8]	*ca.* 0.96 met.-sec. (14.7°)[8]
Mammalian muscle		
Rabbit's gastrocnemius ..	*ca.* 2 σ (*ca.* 37°)[9]	10–13 met.-sec. in man's forearm [10]

tions. The second column gives the time occupied by the rise of the action-current curve from zero to its apex, as measured usually with the thread galvanometer. The third column gives the velocity of propagation of the excitation-wave. The table includes not only observations on various normal tissues at different temperatures, but also a number of observations on narcotized

[4] SNYDER: American Journal of Physiology, 1913, xxxii, p. 336.

[5] HERMANN: Archiv für die gesammte Physiologie, 1877, xv, p. 233; propagation-rate in frog's gastrocnemius determined by Matthias: *ibid.*, 1892, liii, p. 70; his experiments give a range of 3.27 to 6.36 met.-sec.

[6] GARTEN: Winterstein's Handbuch der vergleichenden Physiologie, Bd. III, 1910, p. 113.

[7] K. LUCAS: Journal of Physiology, 1909, xxxix, p. 207. The propagation-velocities are not given by Lucas, but are estimated from his curves.

[8] BABKIN: Archiv für die gesammte Physiologie, 1908, cxxv, p. 595.

[9] GARTEN: *loc. cit.*, p. 114; Zeitschrift für Biologie, 1909, lii, p. 534.

[10] HERMANN: Archiv für die gesammte Physiologie, 1876, xvi, p. 410; MATTHIAS: *ibid.*, 1893, liii, p. 70.

TABLE (*Continued*)

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
B. Nerve:		
Frog's sciatic	0.9–1.2 σ (*ca.* 18°)[11]	20–40 met.-sec. at 20° [12]
" "	0.55 σ (32°)[11]	30–80 met.-sec. at 30° [12]
Rabbit's sciatic	*ca.* 0.5 σ (32°)[13]	*ca.* 100 met.-sec. at 37°
Dog's "	*ca.* 0.7 σ (36°)[13]	*ca.* 100 met.-sec. at 37°
Non-medullated (splenic of horse).....	*ca.* 60–70 σ [14]	*ca.* 0.47–0.54 met.-sec.[14]
Olfactory of pike	*ca.* 70 σ (12°)[15]	60–90 mm.-sec. (5°) 118–150 mm.-sec. (13°) 160–240 mm.-sec. (20°)[16]
Commissural of anodonta.	*ca.* 200 σ [17]	*ca.* 2.5 cm.-sec. (varying estimates from 1 to 5 cm.-sec.)[17]
Mantle-nerve of octopus.	8.2–11.3 σ [18]	2.5–3.5 met.-sec. in O. vulgaris [20]
" "	*ca.* 20 σ [19]	*ca.* 2 met.-sec. in O. punctatus [21]

and fatigued tissues. It will be seen that in all cases the two values show a closely parallel variation in different animals, and also in the same animal under varying external conditions. Tissues whose normal bioelectric variations are slow exhibit slow conduc-

[11] GARTEN: Winterstein's Handbuch, *loc. cit.*, p. 137.

[12] SNYDER: American Journal of Physiology, 1908, xxii, p. 179.

[13] GARTEN: *loc. cit.*, p. 139.

[14] GARTEN: *loc. cit.*, pp. 144–6.

[15] GARTEN: *loc. cit.*, p. 141.

[16] NICOLAI: Archiv für die gesammte Physiologie, 1901, lxxxv, p. 65; Archiv für Anatomie und Physiologie, Physiol. Abth., 1905, Suppl., p. 341.

[17] GARTEN: *loc. cit.*, p. 142.

[18] FUCHS: Sitzungsberichte der kais. Akad. d. Wiss. Wien, 1894, Bd. 103, Abth. 3, p. 207.

[19] BORUTTAU: Archiv f. die gesammte Physiologie, 1905, cvii, p. 193.

[20] GOTCH: Schäfer's Text Book, Vol. II, p. 482; FUCHS: *loc. cit.*

[21] JENKINS and CARLSON: American Journal of Physiology, 1903, viii, p. 262.

TABLE (*Continued*)

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
C. Cardiac Muscle:		
Ventricular muscle of mammal	10–15 σ [22] (body temperature)	Averages apparently 2–4 met.-sec.[23]
Ventricular of frog	40–60 σ [24] (*ca.* 18°)	From 50–200 mm.-sec.[25]
D. Smooth Muscle:		
Retractor penis of dog	*ca.* 2 sec.[26]	1–7 mm. sec.; average *ca.* 5 mm.-sec.[26]
Ureter-muscle	0.2–0.4 sec.[27]	Average *ca.* 14–15 mm.-sec.[27]

tion-rates and *vice versa;* and whatever condition retards the velocity of the local bioelectric process also retards the velocity of propagation.

These data appear to be sufficiently extensive and varied to leave no doubt that a close positive correlation exists between the rate at which the electrical variation rises to its maximum at any region of the excited tissue, and the rate at which the excitation-wave is propagated in that tissue. It is not immediately evident why this should be the case. Conceivably the physiological change

[22] The duration of the upstroke of the prominence in the electrocardiogram called R in EINTHOVEN's terminology. *Cf.* EINTHOVEN: Archiv f. d. gesammte Physiologie, 1908, cxxii, p. 517; KAHN: *ibid.*, 1909, cxxvi, p. 197, cxxix, p. 291; SAMOJLOFF: Archiv für Anatomie u. Physiologie, Physiol. Abth., 1910, pp. 508–9.

[23] BAYLISS and STARLING, SCHLÜTER, WALLER and REID: For references *cf.* NAGEL's Handbuch der Physiologie, i, p. 250.

[24] Estimated from the duration of the upstroke of the ventricular variation R in SAMOJLOFF's electrocardiograms. *Cf.* Archiv für Physiologie, 1910, p. 507; Archiv f. d. gesammte Physiologie, 1910, vol. cxxxv, p. 417; *ibid.*, 1912, cxlvii, p. 249.

[25] ENGELMANN, BURDON-SANDERSON. *Cf.* NAGEL's Handbuch, i, p. 250. The velocity varies of course with temperature.

[26] VON BRÜCKE: Archiv f. d. ges. Physiol., 1910, cxxxiii, p. 313.

[27] ORBELI and VON BRÜCKE: *ibid.*, p. 341.

TABLE (*Continued*)

Tissue	Duration of rising phase of action-current curve (σ = .001 sec.)	Velocity of propagation of excitation-wave
E. Influence of Narcotics: [28]		
Frog's sciatic	Normal: 3.2–4 σ (8.9°); partly narcotized: 3.4–4.4 σ [29]. Normal *ca.* 4 σ; narcotized 5.2–6σ [30]	Average 17.3 met.-sec. in normal; *ca.* 12 met.-sec. in weakly, 9.4 met.-sec. in more strongly narcotized nerves.[29]
Pike's olfactory.....	Normal 55–60 σ (*ca.* 9°)[29] partly narcotized: 67–82 σ	Average normal velocity: *ca.* 81 mm.-sec.; in narcosis *ca.* 59 mm.-sec.[29]

determining propagation might be quite independent of the bioelectric process,[31] just as, for example, it appears in nerve to be independent of heat-production. The above evidence, however, plainly points to the existence of a close connection between the two. Garten, Fröhlich, and Lucas have pointed out this parallel-

[28] Fatigue also decreases both propagation-velocity and rate of electrical variation in muscle. *Cf.* VON BRÜCKE: Archiv f. d. ges. Physiol., 1908, cxxiv, p. 215. This is probably the case whenever an impulse is conducted with a decrement.[30] FUCHS also notes the lengthening of the action-current curve in the fatigued nerves of cephalopods (*loc. cit.*).

[29] KOIKE: Zeitschrift für Biologie, 1911, lv, p. 311. It will be noted that according to Koike's curves the duration of the rising phase in the frog's nerve is only slightly decreased during narcosis; the slope of the curve, however, and hence its total height, are much lower (two-thirds or less) than in the normal nerve; and the retardation of propagation-velocity is probably due chiefly to the decrease in the amplitude of the variation.

[30] FRÖHLICH: Zeitschrift f. allg. Physiologie, 1912, v, xiv, p. 55. BORUTTAU and FRÖHLICH (Archiv f. d. ges. Physiol., 1904, xv, p. 444) also show that both the rate of rise and the amplitude of the electrical variation are decreased during narcosis.

[31] ELLISON has in fact recently maintained that the electrical variation can be abolished in nerve by cinchonamine hydrochloride without interfering with conduction (Journal of Physiology, 1911, xliii, p. 28); but his work has not received confirmation. *Cf.* DITTLER and SATAKE: Archiv f. d. ges. Physiol., 1912, cxliv, p. 229; also LUCAS' critique, Proc. Roy. Soc., B. 1912, lxxxv, p. 503.

ism,[32] but without attempting an analysis of its exact significance. Such an analysis is evidently necessary to any complete consideration of the nature of the processes of stimulation and conduction, and I shall accordingly attempt it in what follows.

It is possible to explain this correlation in two essentially different ways. First, it may be supposed that the rate of the rise and subsidence of the electrical variation at any point of the conducting tissue is simply an index of the time which the excitation-wave takes to pass that point. This view regards the electrical variation as a mere accompaniment or sign of an underlying process which is transmitted in the form of a wave along the tissue. Obviously the more rapidly this wave passes a given point the more rapidly the associated bioelectric process will rise to its maximum and subside at that point. On the other hand, it is possible that the electrical variation is itself the essential feature of the excitation-process, and constitutes that functional component of the local process which directly excites the adjoining regions of the tissue to activity; if so, its rate of development must determine the rate at which excitation is transmitted from the active region of the tissue to those adjoining. This possibility has been hitherto largely disregarded; it is, however, consistent with all that we know of the conditions of stimulation, and recently the evidence in its favor has greatly increased. As I shall attempt to show below, this view accounts satisfactorily both for the transmission of the excitation-wave, and for the wide variation in the rate of this transmission in different tissues.

It should be pointed out that according to this view not only the rate of development or time of rise of the local electrical variation but also its total amplitude (or voltage) is a factor in determining the rate of conduction. This amplitude is, however, the less variable of the two factors; and hence the correlation between the propagation-velocity of the excitation-wave and the rate of development of the action-current is much more clearly evident than that between propagation-velocity and amplitude. But in some cases, as in Koike's observations with narcotized nerves cited above, the chief factor in reducing the propagation-

[32] GARTEN: Winterstein's Handbuch, *loc. cit.;* FRÖHLICH: *loc. cit.;* LUCAS: *loc. cit.*

velocity appears to be the decreased range of the variation rather than the decreased duration of its rise. This period is not greatly shortened in the experiments of Koike, while the height of the action-current curve is reduced to two-thirds or less of its normal value. In the majority of instances, however, the rate at which the variation rises to its maximum is apparently the essential or preponderant factor in determining the rate of conduction.

As already said, this correlation is readily intelligible if the transmission of the excitation-wave is a direct consequence of the electrical variation as such. If we suppose that for transmission of excitation from the already active region of the nerve to an inactive region 2 centimeters (*e.g.*) distant the existence of a certain minimal potential-difference between active and inactive regions is required, *e.g.* 20 millivolts, then the more rapidly this potential-difference is attained the more rapid will be the transmission. Now in point of fact a current between platinum electrodes 2 centimeters apart differing in potential by 20 millivolts or even less is amply sufficient to excite a sensitive nerve. If we assume that the voltage of the action current is 30 millivolts, then the current flowing along the nerve between these two electrodes is approximately equal to that flowing between a region, A, already active (and therefore negative) and an inactive region, B, 3 centimeters distant. If this action-current, at the instant when it reaches its maximum, causes excitation at this distant point B, and the resulting negative variation there reaches its maximum in .001 second, a second excitation-impulse will be transmitted from B to a point 3 centimeters further along the nerve in the same time, .001 second. Three centimeters in .001 second is in fact the approximate velocity of the nerve impulse in frog's nerve at 20°. It thus appears possible that the velocity of propagation is a function (1) of the rapidity with which the excited region undergoes its electrical variation, — *i.e.*, assumes externally a certain negative potential relatively to the unexcited regions, and (2) of the maximal distance along which the current passing between this temporarily negative region and the as yet unexcited (positive) regions of the nerve can make itself felt as a stimulus. This distance will depend on a number of variable conditions, — the amplitude of the electrical variation, the electrical resistance of

the tissue, its threshold of stimulation, the rate of rise of the stimulating current to its full intensity, the electrical condition of the nerve in the intermediate region. According to this hypothesis, in the time t (in seconds) after stimulation there would be electrical stimulation of the nerve at a point B, s centimeters distant from the already active region A; point B would then become after the same interval the source of stimulation for a third point, C, s centimeters beyond, and this process would continue. The velocity of propagation in centimeters per second would then be measured by the quotient s/t, where t is the time between stimulation and the attainment of a certain critical point in the action-current curve, and s the maximal distance at which the current between active and as yet inactive regions just suffices to stimulate.

It will be noted that this view takes no account of the differences in the intensity of the electrical stimulus imparted by the action-current to the nerve at different distances from the active region. There is, however, strong evidence that the intensity of the electrical stimulus is a matter of indifference in the stimulation of a normal nerve, provided the stimulus reaches the threshold value: *i.e.*, that the "all-or-none" law applies to the normal nerve fibre.[33] Hence any stimulus that excites at all will call forth a response of full intensity. The *maximal* distance from the active region at which the branch of the action-current causes excitation is thus the only one to be considered. For transmission of the above conceived kind it is in fact essential that the "all-or-none" law should apply at all points of the nerve. Under other conditions the impulse would inevitably die out,[34] as in fact appears to be the case in a narcotized stretch of nerve. It appears highly probable that the "all-or-none" form of local response is indispensable to any tissue whose activity depends on conduction of the excitation-state to some distance from the point of stimulation.

[33] *Cf.* GOTCH: Journal of Physiology, 1912, xxviii, p. 395; VESZI: Zeitschrift f. allgemeine Physiologie, 1912, xiii, p. 321; ADRIAN: Journal of Physiology, 1913, xlv, p. 321; LODHOLZ: Zeitschrift für allgemeine Physiologie, 1913, xv, p. 269.

[34] Compare my discussion of the mechanism of transmission in my former paper on the present subject: American Journal of Physiology, 1911, vol. xxviii, pp. 217 *seq.*

On the present view, therefore, normal physiological stimulation is identical with electrical stimulation, and the spread of the excitation-state depends directly upon the bioelectric variation accompanying the local stimulation, the velocity of this spread being a function (1) of the sensitivity and electrical conductivity of the tissue and (2) of the amplitude and time-relations of the bioelectric variation. Numerous biological facts are in harmony with this hypothesis besides those already cited, and I shall now proceed to review briefly what seem to be the most significant of these.

It has been known since the work of Matteucci and Du Bois-Reymond that the electrical variation accompanying the activity of one irritable tissue can stimulate another tissue. An active frog's muscle can thus stimulate another muscle or a nerve; the action-current of the ventricular beat can stimulate a sensitive nerve muscle preparation, and so on. There are limits to the possibilities of this kind of excitation; ordinarily an active nerve does not stimulate a muscle, and the action-current of smooth muscle will not stimulate a nerve. Apparently there is needed a certain correspondence between the time-relations of the electrical variation in the stimulating tissue, and the time-factor of electrical excitation in the responding tissue. The rise of the electrical variation in the one tissue may be too gradual to stimulate another tissue which requires a rapid rate of change in the stimulating current; or its duration may be too brief, even though its rate of development and intensity may be sufficient. Thus the electrical variation of the frog's ventricle may stimulate a nerve [35] (especially one cooled so as to prolong its time-factor of excitation), but that of the nerve is too brief to stimulate this kind of muscle. There is thus here a certain relation of irreciprocality in the transmission,

[35] KÜHNE found the action-current of the turtle's heart (*Testudo græca*) incapable of stimulating a very sensitive frog's nerve, though able to excite the curarized frog's sartorius. On the other hand, the action-current of the rabbit's heart proved very effective as a stimulus to frog's nerve. Kühne rightly ascribes the difference to the difference in the time-relations ("zeitliche Verlauf") of the action-currents of the two hearts. Similarly with the failure of the action-current of smooth muscle to stimulate nerve. *Cf.* KÜHNE: Untersuchungen aus dem physiologischen Institut der Universität Heidelberg, 1879, Bd. iii, pp. 55, 87 *seq.*

— which suggests a possible basis for the irreciprocality of conduction between motor and sensory nerve-cells in the reflex arc.[36]

Under appropriate conditions the transmission of the excitation-state from one irritable element to another by means of the former's action-current takes place with perfect constancy. Now conduction means simply the excitation of one region of a continuous element by some process occurring in the adjoining active region; and we must therefore examine more closely the possibility that the electrical variation is the essential change that causes this excitation. The action-current is the only known change accompanying excitation in nerve which is competent to stimulate an adjoining stretch of nerve, and it is known that under certain conditions one nerve may in fact be stimulated by the action-current of another.[37] There is also good evidence that the character and intensity of the local excitation-process undergo no essential change as it passes along nerve or muscle fibres. At the point of external stimulation the process has the same characteristics as in regions that enter into activity by "conduction" from adjoining excited regions.[38] It is clear that the local excitation-process includes some functional component which acts as stimulus on adjoining regions and produces there the same effect as does the external stimulus. That this component is the electrical variation is indicated not only by the above general considerations but by various facts of comparative physiology to be partly cited below.

Before citing these facts it seems desirable to consider the

[36] The action current of a rapidly responding tissue (or cell) may not last long enough to stimulate a slowly responding tissue; while that of a slowly responding tissue is always sufficient, as regards mere duration, to stimulate the rapidly responding; the slowly rising is less effective than the rapidly rising action-current only if its rate of rise is so gradual that the requisite current-strength is not attained within the characteristic time-period of the responding tissue. Similar considerations of course apply to the interactions between adjacent cells. See below, p. 439.

[37] HERING: Sitzungsberichte d. Akad. Wiss. Wien, math-naturw. Kl., 1882, Vol. lxxxv, 3te Abth. p. 237; V. UEXKÜLL: Zeitschrift für Biologie, 1894, xxx, p. 184 *ff*. *Cf*. also BIEDERMANN: Electrophysiology, English translation, Vol. 2, pp. 264 *seq*.

[38] *Cf*. LUCAS: Journal of Physiology, 1906, xxxiv, p. 51; BRAMWELL and LUCAS: *ibid*., 1911, xlii, p. 495. The propagated disturbance is associated with a refractory period similar in all respects to that at the site of stimulation.

alternative hypothesis that some chemical or "molecular" change forms the direct basis of transmission. This hypothesis appears to be a favorite with many physiologists, but it has the disadvantages both of being vague in itself and of having no satisfactory analogues in inorganic processes. The rapid transmission of chemical effects, as in an explosion, is seen on closer analysis to be a secondary consequence of the transmission of local changes of temperature or pressure, or both combined. In a train of gunpowder, for instance, the rate of transmission depends mainly on the rate of conduction of heat between the ignited and the adjoining unignited areas, combustion beginning when the temperature reaches the ignition-point. In other explosions additional factors enter: *e.g.*, the mechanical shock resulting from the local reaction, and the development of pressures which are transmitted with high velocity; such transmission may be much more rapid than that depending on local rise of temperature alone.[39] It is obvious that in such a tissue as nerve, where mechanical and thermal changes are absent or insignificant, these factors cannot enter. The only other known local process that can serve as a basis for the transmission is the electrical one. It is significant that in the cases of "chemical action at a distance" described by Ostwald [40] the basis of transmission is electrical; this is also the case in the spread of the catalytic action over the surface of the mercury in the rhythmical hydrogen peroxide catalysis of Bredig; according to Antropoff,[41] this transmission is due to electrolytic action at the boundary of the surface-film of mercury peroxidate; this action automatically dissolves the film at that region, and the process thus initiated then spreads over the entire surface. I have already pointed out some of the many remarkable analogies between this process and

[39] The high velocity of the explosion-wave in guncotton, gases confined in tubes (where it may exceed 2000 meters per second), etc., is due to the development and rapid transmission of high pressures, with accompanying generation of heat. *Cf.* WILL: Zeitschrift für Elektrochemie, 1906, xii, p. 558. Also NERNST's Theoretical Chemistry and MELLOR's Chemical Statics and Dynamics for a more general account of explosive action.

[40] OSTWALD: "Chemische Fernwirkung": Zeitschrift für physikalische Chemie, 1891, ix, p. 540.

[41] ANTROPOFF: Zeitschrift für physikalische Chemie, 1907, lxii, p. 513.

the processes of physiological excitation and conduction.[42] The progressive dissolution of the film of peroxidate covering the mercury is a good instance of that kind of chemical action which depends on conditions essentially characteristic of surfaces. The potential-difference between the film-covered mercury-surface and the adjoining peroxide solution differs from that between the free metallic surface and the solution by *ca.* 0.12 volts; hence when the free mercury surface is exposed — *e.g.*, by local mechanical rupture of the film — a current flows between these two regions, which exerts electrolytic action and reduces the film to the condition of metallic mercury. Such an effect necessarily spreads, at a rate depending on the velocity of the chemical or electrolytic process by which the film is locally removed (or sufficiently altered). Similarly the potential-difference between the living cell and its surrounding medium depends on the characteristics of the protoplasmic surface-film; and when this is altered locally — *e.g.*, when its permeability is increased — the potential-difference at that point is also altered and a current flows between the altered and the unaltered regions. This current, on the present hypothesis, is the condition of propagation of the surface-change that determines excitation. The processes on which the propagation depends are thus closely similar as regards determining conditions in the two cases, although the comparison must of course not be pushed too far into detail.[43]

In addition to the experimental facts already cited, there is much general biological evidence that the transmission of excitation in many other living tissues and cells is due to the electrical variations of the active elements. One muscle may be stimulated by the action-current of another; for such transmission contact or close proximity of the tissues is necessary, but not direct continuity of irritable elements.[44] Transmission of excitation from

[42] *Cf.* American Journal of Physiology: 1909, xxiv, p. 18.

[43] Du Bois-Reymond concluded — from the fact that electrical stimulation is a polar phenomenon — that the primary effect in stimulation is an electrolysis. *Cf.:* Untersuchungen über thierische Electricität, vol. ii, p. 387: "Galvanische Reizung ist uns nichts mehr als die erste Stufe der Elektrolyse eines Nerven." Compare the suggestion made below, p. 444.

[44] *Cf.* Kühne: Croonian Lecture, *loc. cit.* p. 446: "Two (curarized) muscles . . . need only be pressed together transversely over a narrow area to make a

cell to cell without any organic continuity is in fact a widespread phenomenon, and one difficult to explain except as the result of electrical excitation by the action-current. There is the case of transmission from neurone to neurone across the synapses; but disagreement still exists as to whether the interlacing dendrites are continuous or not, so that this instance might be regarded as equivocal. The propagation of waves of increased activity over the surface of a ciliated epithelium is perhaps a better instance; increased ciliary activity in one area excites adjoining areas to increased activity, so that a certain synchrony tends to be preserved between neighboring cells. If ciliary activity, like other forms of contractility, is due to variations of electrical polarization at the surfaces of the contractile elements,[45] an action-current must accompany each ciliary stroke, and its stimulating influence will be transmitted through the medium for some distance. Some observations which I made at Naples a number of years ago,[46] on the swimming-plates of the ctenophore *Eucharis*, support this view: the successive plates of a single row beat in order, so that waves of movement continually run along the row from the aboral to the oral end; if a strip is cut from the body of the animal, containing a row of plates together with a portion of the underlying jelly, and the piece of tissue is left for some hours in sea-water, the mass of jelly contracts and rounds off, and frequently in such a way that the opposite ends of the row are brought within a few millimeters of each other. The rounded portion of jelly is then encircled by a row of still active plates. When this condition is reached it frequently happens that the wave of ciliary movement is transmitted across the interval between the opposite ends of the row, so that the movement continues to travel round and round the ring of tissue in the same direction, often for hours at a time.

single muscle of them of double length in which the stimulation and contraction are propagated from one end to the other. Since the transference from one muscle to the other is done away with as soon as we bring the finest gutta percha between the muscles as an insulator, or gold leaf as a secondary circuit, the first muscle must have excited the second electrically."

[45] *Cf.* my paper on the conditions of activity of the ctenophore swimming plate, in American Journal of Physiology, 1908, xxi, p. 214 *seq.*

[46] Briefly reported in the Year-Book of the Carnegie Institution, No. 4, 1905, p. 282.

The interval across which the stimulus may thus be transmitted may be half a centimeter or more. The time is too short for regeneration of a special conducting tissue (supposing this to exist); the exciting influence is evidently transmitted through the sea-water and jelly; and nothing but an electrical influence is known to act in this manner. Another striking instance of transmission of excitation without organic continuity between the active elements is furnished by the spermatozoa of certain marine animals, especially of the annelid *Nereis*. The spermatozoa of *Nereis*, if collected in watch-glasses, gather together into numerous small groups or aggregations shortly after shedding from the body of the animal. After standing for a few minutes such an aggregation settles down into a layer, and it is then found that the separate spermatozoa forming this layer exhibit a marked tendency to beat in unison with one another; a regular and synchronous beating thus becomes established, the whole group of cells resembling in this respect a ciliated epithelium; and waves of accelerated movement are transmitted from cell to cell just as in such an epithelium.[47] It is difficult to conceive of any influences other than the electrical which can cause these cells thus to transmit their rate of movement to one another; if, however, each stroke of a flagellum is accompanied by an action-current whose influence extends for a short distance through the surrounding sea-water and is sufficient to stimulate neighboring spermatozoa, the phenomenon becomes at once intelligible.

The facts just cited afford evidence that the excitation can be transmitted from one irritable element to another through the surrounding medium independently of any organic connection between the elements. The media of irritable tissues, however, characteristically contain salts, and the withdrawal of these is well known to arrest activity in many cases, especially in muscle

[47] F. R. Lillie: Journal of Experimental Zoölogy, 1913, xiv, p. 523. There is also evidence that adjacent blastomeres undergoing mitosis influence one another, so that a certain synchrony tends to be maintained, which disappears when the cells are separated. *Cf.* M. Sorokina: "Uber Synchronismus der Zelltheilungen," Archiv f. Entwicklungsmechanik, 1912, xxxv, p. 30. If cell-division is accompanied by changes in the electrical polarization of the cell-surface, such transmission is readily explained in the present hypothesis.

and nerve, — tissues in which it is essential that the local disturbance should be propagated for some distance if the irritable element is to react as a whole. Brünings has attributed part of the action of sugar solutions in temporarily depriving muscle and nerve of their normal properties to a lowering of the electrical conductivity of the media;[48] if the propagation of the excitation-impulse depends on this condition, it is easy to see why irritability is temporarily lost in solutions of non-conductors. Under normal conditions the electrical variation which accompanies activity may be conducted for a considerable distance through the medium with an intensity sufficient for stimulation. The precise degree to which this is possible depends on the electrical conductivity of the medium as well as on several other factors, chief of which are the sensitivity of the irritable elements to electrical stimulation, and the intensity and time-relations of the electrical variation. When electrical conductivity is lowered to a sufficient degree, transmission of excitation is no longer possible.

The idea that organic continuity is necessary for the transmission of excitation is derived more especially from the conditions in nerve, where the conducting elements are typically continuous, and where severing this continuity prevents the passage of the impulse. But that transmission is possible without this continuity is sufficiently clear from the facts already cited. There are, however, advantages in continuity of the kind shown by nerve-fibres if the conduction is to be *rapid*. The presence of cross-partitions at intervals would decrease the electrical conductivity of the tissue in the longitudinal direction and thus lessen the distance along which the local electrical effect could be transmitted with an intensity sufficient for excitation. According to Hermann the electrical resistance of frog's nerve in the direction *across* the fibres (*i.e.*, with partitions — or surface-films — interposed) is five times as great as in the lengthwise direction.[49] There is in fact

[48] *Cf.* BRÜNINGS: Archiv f. d. gesammte Physiologie, 1907, cxvii, p. 418.

[49] HERMANN: Archiv f. d. gesammte Physiologie, 1872, v, p. 223. The same is true of muscle. In dead muscle and nerve this difference disappears or is greatly diminished. HERMANN refers the resistance to the presence of polarizable elements or membranes in the tissues; this polarizability largely disappears on the death of the tissue. Polarizability, as we now know, is a function of semi-permeability, which is lost at death.

a marked decrease in the velocity of conduction of the nerve-impulse between different neurones, a condition quite possibly due in part to the interposition of the relatively impermeable and hence non-conducting surface-films of the adjoining synapses.

We have now to consider the question: are the characteristics of the local electrical variation (*e.g.*, in nerve) such that excitation through its means of an inactive region of nerve situated at some distance from the active region (of at least 2 or 3 centimeters) is possible? In order that an electric current traversing an irritable tissue may cause excitation it must have a certain minimal strength, a certain minimal duration of flow, and a certain minimal rate of change of intensity. We have therefore to inquire whether that portion of the action-current which traverses the nerve at the above distance from the immediate site of activity has these characteristics.

First, with regard to the intensity of the current flowing between excited and unexcited regions. The usual law relating current-strength to voltage and resistance will apply. The intensity of the current between excited and inactive regions will thus decrease as the distance between the two regions increases, being at any point inversely proportional to the electrical resistance between that point and the excited region.[50] The current will thus be strong near the site of activity, decreasing as the distance from this increases; there will thus be a distance beyond which it will be ineffective as a stimulus. The question to be answered is: what is this maximal distance? The problem can only be approached indirectly. The following considerations seem to point the way to an approximate solution. The electromotive force of the action-current of nerve is approximately equal to that of the demarcation-current (*ca.* 0.03 volt in frog's nerve). Now the length of the stretch of nerve by traversing which the demarcation-current can actually cause excitation may be very considerable, in favorable cases 2 centimeters or more.[51] This is shown by the well-

[50] This resistance will in a cylindrical nerve be directly proportional to the distance.

[51] HERING obtained twitches with a distance of 25 millimeters between the clay pads across which the nerve lay. The nerve current was closed by means of a vessel containing salt solution brought up from below. *Cf.* HERING: Sit-

known experiment where the resistance of the external portion of the demarcation-current circuit is suddenly decreased or increased by connecting cross-section and longitudinal surface by a wire or (in Du Bois-Reymond's arrangement) a bridge of filter paper or clay soaked in salt-solution. In this experiment what is really done is suddenly and greatly to change the resistance in the external part of the circuit through which the nerve-current is flowing; this of course is equivalent to suddenly increasing or decreasing the flow at all points of the circuit; so that the effect of suddenly applying or withdrawing the external conductor is really that of cross-circuiting or the reverse, *i.e.*, is the same as if the demarcation-current were to be suddenly closed or opened. The maximal distance which may separate the points of contact at cut and longitudinal surfaces without annulling the stimulating action of the demarcation-current may thus be regarded as closely corresponding with the maximal distance along which the demarcation-current could make itself felt as stimulus if it were suddenly to come into existence or disappear. Now the normal effect of excitation is to produce rapidly a local negativity like that at the cut surface; the electrical effects at neighboring regions of the nerve must therefore be the same as in the case just considered. Hence we infer that the action-current, on its sudden local appearance following stimulation, itself stimulates electrically the nerve at a distance from the locus of excitation at least equal to the maximal distance through which the demarcation-current may stimulate in the above experiment, — *i.e.*, from 2 to 3 centimeters. In the intact animal the maximal distance through which the stimulating action extends is probably greater than this, since in the excised nerve the conditions are necessarily more or less abnormal, and local potential-differences at the cut ends of side branches or at points of injury will give rise to minor demarcation-currents which will interfere with and partially compensate the action-current, thus decreasing its stimulating effect. It is well known that in the experiment where the nerve is stimulated by its own demarcation-current as the result of being dropped suddenly on another conducting surface (a piece of tissue or a pad of clay

zungsberichte d. kais. Akad. Wiss. Wien, math.-naturw. Kl., 1882, lxxx$_V$, Abth. 3, p. 240.

moistened with salt-solution), it is highly important that the latter should be "isoelectric," since otherwise interferences arise which impair the success of the experiment. In the above experiment with the demarcation-current the external conditions are always more or less unfavorable to stimulation by weak currents, and we may infer that in the intact animal the action-current, when it arises at any point in the nerve, exerts a stimulating effect at a greater distance from this point than the maximal distance separating the electrodes in Du Bois-Reymond's experiment. How much greater is difficult to say on *a priori* grounds, and any direct experimental determination seems impracticable.

Another indirect method of attacking the present problem is to determine the maximal distance that may separate electrodes having the same potential-difference as that between active and inactive regions (20 to 30 millivolts in nerve) without preventing stimulation; *i.e.*, to determine the potential-difference between electrodes a known distance apart when the current has an intensity just sufficient for stimulation. We may in fact regard excited region and unexcited region as two areas of different potential united by a conductor. The current flowing between these regions, if sufficient in intensity and duration, will excite the nerve along the line of flow. The stimulating effect will be the same as if the two regions were electrodes differing in potential by a certain degree.

Recently I have made a large number of determinations of the least potential-difference required for stimulating the frog's sciatic nerve through platinum electrodes separated by a stretch of nerve varying from 1 to 2 centimeters in length. These experiments have shown that while the sensitivity of the nerve to electrical stimulation varies considerably in different nerve-muscle preparations, yet in sensitive nerves currents of 10 to 15 millivolts P.D. frequently cause well-marked stimulation with the electrodes 15 or 20 millimeters apart, and not infrequently stimulation occurs with much weaker currents.

The following is an instance of an experiment with a somewhat more than usually sensitive pair of nerves. The electrodes were platinum wires of about 0.3 mm. diameter; these were connected through a rocking key or pole-changer to the zero post and slider

of an Ostwald meter-wire rheocord. The rocking key was also connected with a Weston millivolt meter, so that (in the absence of the cross wires) the E.M.F. of the current supplying the electrodes could be read off directly by turning the rocker. The rheocord was connected with a single Edison normal cell. The nerve was suspended between electrodes 15 millimeters apart.

The following figures give the potential difference (millivolts between the electrodes) of the weakest current that caused definite contraction of the leg or foot muscles on make or break of the current. Both sciatic nerves of the frog (*Rana virescens*) were used with both ascending and descending currents.

	Direction of current	Voltage of weakest current
Nerve 1	Descending	10 mv. (stimulation at make, not at break)
"	Ascending	13 mv. (stimulation at break, not at make)
Nerve 2	Descending	9 mv. (stimulation at make only)
"	Ascending	10 mv. (stimulation at break only)

The following is a record of a similar experiment with a less sensitive pair of nerves.

	Direction of current	Voltage of weakest current
Nerve 1	Descending	22 mv. (stimulation at make only)
"	Ascending	28 mv. (stimulation at break only)
Nerve 2	Descending	20 mv. (stimulation at make only)
"	Ascending	22 mv. (stimulation at break only)

Preparations are not infrequently more sensitive than either of the above, and occasionally stimulation occurs with a P.D. of only 5 or 6 millivolts. This extreme sensitivity is most frequently met after the nerve has been exposed to the air for a time and has begun slightly to dry. Usually such marked increase of irritability is followed by spontaneous twitching of the muscle; bathing the nerve in Ringer's solution then arrests the twitching.

With the electrodes 2 centimeters apart the minimal voltage is somewhat higher, but is frequently so low as 10 to 15 millivolts, and in some cases I have obtained stimulation with 8 millivolts.

It has been my experience that the make and the break of these weak constant currents are almost equally effective as stimuli. All that appears to be needed is the rapid establishment of a certain minimal potential-gradient along the nerve. The above observations show that this gradient may be so low as 10 millivolts in 2 centimeters, which is the same as 30 millivolts (the approximate variation of potential in the action-current) in 6 centimeters. It might possibly be objected that the nerve was in a hypersensitive condition in these experiments, or that the observed stimulation was really the result of a summation of the effects of the external current and of local nerve-currents due to drying or injury. Observation shows, however, that the voltage of the minimal exciting current tends toward an approximately constant lower limit when the electrodes are a definite distance apart, — a fact indicating the existence of a definite threshold-intensity for a current traversing the nerve. This intensity is not greater than that of a current flowing between two regions of a nerve several centimeters apart differing in potential by 20 or 30 millivolts, the approximate E.M.F. of the action-current of frog's nerve.

We conclude that the current flowing between excited and unexcited regions of a normal frog's motor nerve is sufficiently intense at a distance at least $2\frac{1}{2}$ or 3 centimeters from the excited region to cause stimulation at that point.

The question of whether its duration is sufficient may readily be answered, since we have recent accurate determinations by Lapicque and Lucas of the least effective duration of currents of different strengths, — ranging from the weakest currents that will excite with any duration of flow, to currents whose least effective duration is a small fraction of that of the weakest exciting current. Within the range of conditions to which Nernst's rule applies, the current-strength required for stimulation varies approximately inversely as the square root of the duration. From this it is evident that a current of briefer duration than the rising phase of the action-current (*ca.* 0.001 second at 20°) may stimulate a nerve if its intensity is sufficient. The question is, whether the current flowing between the region already active and an inactive region 2 or 3 centimeters distant is sufficiently intense to stimulate with a duration not greater than that of the rising phase of the action-

current. Lapicque has shown that the minimal current that will stimulate a nerve with infinite duration of flow requires for stimulation a duration not much greater than that of the rising phase of the action-current; and in order to stimulate with a duration not greater than this (*i.e.*, *ca.* .001 sec.) the intensity needs to be increased to only a relatively slight degree (respectively 33 per cent and 50 per cent for the two experiments at 24.5° and 18° which he cites).[52] This general rule holds true not only of nerve but of other irritable tissues, and probably of irritable tissues in general.[53] The time occupied by the rising phase of the action-current is in fact closely similar to the time during which a current of threshold-intensity requires to flow in order to stimulate. This time is characteristic for any irritable tissue, and constitutes what Lapicque calls the chronological factor in electrical excitation.[54] It varies from tissue to tissue in a manner which shows close parallelism with the characteristic time-relations of the bioelectric variation, its exact duration in any tissue being closely similar to — though apparently usually somewhat greater than — that of the rising phase of the electrical variation.[55] It is thus clear that the action-current lasts quite sufficiently long to stimulate the adjoining areas of the irritable tissue at a distance not much less than the maximal distance at which (on the above estimate) its intensity is sufficient.

In order to simplify our conception of the conditions as much as possible, let us regard the region actually undergoing excitation as stationary; it is evident that the electrical effect at any point

[52] *Cf.* LAPICQUE: Journale de physiologie normale et pathologique, 1907, ix, p. 628; *ibid.*, 1908, x, p. 599. The conditions are the same at lower temperatures; here, of course, the duration of the action-current is proportionately prolonged.

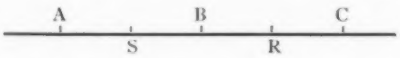
[53] *Cf.* the observations of LUCAS on the frog's sartorius, Journal of Physiology, 1908, xxxvii, p. 475, and of LUCAS and MINES on the sciatic and sartorius of the toad, *ibid.*, 1907, xxxvi, p. 334.

[54] *Cf.* LAPICQUE: Journale de physiologie, 1908, x, p. 601.

[55] *Cf.* LAPICQUE, *loc. cit.;* also *Cf.* LUCAS: Journal of Physiology, 1910, xxxix, p. 461. According to Lucas the normal rate of change characteristic of the excitatory process in a particular tissue is what determines its time-factor of electrical stimulation. Hence this is brief in rapidly reacting tissues like nerve, etc.

beyond this active area will vary in intensity during the time occupied by the entire negative variation at the excited region, and that it will have the requisite intensity for stimulation at that point during only part of the time occupied by the whole variation. If we consider the intensity alone, it is clear that the effect will "reach" farthest — *i.e.*, will be sufficient at a certain distant point as regards mere intensity — when the action-current curve is at its apex; but the *duration* of the branch of the action-current traversing this most distant point will probably be insufficient for excitation. There will, however, be an intermediate point or region where the current is just intense enough and lasts just long enough to stimulate; this region will then at once become the site of a new excitation-process which will produce similar effects at an equidistant point beyond. If the distance of this point from the excitation-area is s centimeters, and the time required for the local excitation-process to reach the required stage for excitation at this point is t seconds, the state of excitation will be propagated with the velocity s/t centimeters per second. With $s=3$ centimeters and $t=.001$ second, the propagation-velocity will be 30 meters per second.

Naturally the progress of the excitation-wave is continuous and does not occur on a succession of leaps, as the above simplified manner of conceiving the phenomenon would seem to imply. This apparent implication, however, merely results from a neglect to consider the processes at other than the two points S, considered as the stimulating point of the nerve, and R, considered as the responding point. Needless to say, the region actually undergoing the changes accompanying excitation occupies a considerable length of the nerve (*ca.* 30 mm. in a single impulse). The actual process would be better described as follows: traveling in advance of the region already active, A B, there is a region B C as yet



inactive but through which a branch of the action-current is flowing; at a certain critical distance B R beyond the active region this current will have the intensity, duration, and rate of development necessary to stimulate the nerve at that point (R). The

greater this critical distance, and the more rapid the local excitation-process, the more rapid will be the rate of propagation.

It is necessary also to consider whether the rate of change of this branch of current is sufficient for excitation. The action-current does not appear suddenly, but rises continuously to a maximum and subsides at a rate that varies characteristically from tissue to tissue. Any stimulating current must not only have a sufficient intensity and duration, but must rise to its stimulating value with sufficient rapidity. The rate of change of the action-current in nerve is, however, actually such that its stimulating efficiency is little if any less than it would be if the current were to rise to its maximum instantaneously. This is shown by Lucas' experiments in the sciatic nerve of the toad, which show that a current which takes .03 seconds to rise to its maximum requires to be only about 20 per cent stronger than an instantaneous current of the same stimulating value.[56] Although the time-factor of excitation in the frog's nerve is somewhat shorter than in the toad's nerve, there is no doubt that a current rising to its maximum in a period 20 to 30 times briefer would have essentially the same effect as an instantaneous current. The rate of change of the stimulating current is related to that of the action-current of the tissue in the same manner as the duration of the least exciting current is related to that of the action-current.[57] A tissue with a brief time-factor of excitation requires a rapidly changing current for stimulation, and *vice versa*. We conclude, therefore, that the rate of change of the action-current in any tissue is adequate for the stimulation of that tissue. The slowly rising electrical variation of a slowly responding tissue is amply capable of stimulating that tissue; although it may be unable to stimulate another tissue with a more rapid rate of response.

We may summarize the foregoing briefly as follows: the facts and considerations adduced indicate that at some distance (probably about 3 centimeters in frog's nerve) from the region already in a state of excitation the branch of the action-current traversing the tissue is sufficient in intensity, duration, and rate of change to stimulate the tissue at that point and hence to initiate a new state

[56] LUCAS: Journal of Physiology, 1907, xxxvi, p. 253.

[57] *Cf.* LAPICQUE: *loc. cit.;* LUCAS: *loc. cit.*, 1908.

of excitation; this in its turn originates a similar excitation at an equidistant point beyond, and so on. Thus the excitation-state is self-propagating by reason of the electrical variation by which it is accompanied, and the rate at which this electrical variation rises to its maximum is the chief factor in determining the velocity of conduction.

This view accounts not only for the propagation of the excitation-wave and for the varying rate of propagation in different tissues, but also for certain other hitherto unexplained peculiarities of irritable tissues. As already pointed out, it affords a possible basis of explanation for irreciprocality of conduction, if it is assumed that the time-factors of excitation of the contiguous neurones are sufficiently different. It also assists in explaining Carlson's generalization that rapidly contracting muscles typically have rapidly conducting nerves, and *vice versa*.[58] In this case we have an instance of transmission of the excitation-state from one irritable element, the nerve, to another, the muscle.[59] On the present hypothesis the muscle cell is stimulated by the electrical variation of the nerve where it branches over the surface of the muscle at the end-plate.[60] The process of transmission from element to element is thus of the same nature as from one region of a continuous element (*e.g.*, nerve) to another. We have seen that for stimulation of the adjoining inactive region it is necessary that the electrical current passing between the active region A B and the inactive B C should have a certain minimal intensity and duration. The intensity is a function of the distance — *i.e.*, electrical resistance — between the two regions; the duration depends on the duration of the local electrical variation at region A B, — or more

[58] CARLSON: *loc. cit.*

[59] The existence in some cases of an intermediary element, the nerve end-plate, with a characteristic excitation-rate of its own, does not essentially alter the conditions.

[60] This view recalls the "Entladungshypothese" put forward by KRAUSE and KÜHNE, in 1863 and 1864 (*Cf.* HERMANN'S Handbuch, i, p. 258), and later criticized, though accepted in a modified form, by DU BOIS-REYMOND. The latter favored the view that the negative variation of the nerve directly stimulated the muscle; this view HERMANN regards as identical with his own view (first put forward in 1872) that the electrical variation is the basis of propagation.

precisely on the duration of that portion of it which has reached a sufficient intensity to affect region B C. If this duration is insufficient, region B C will not be affected and the impulse will fail to be propagated. The same considerations apply to transmission of excitation between separate irritable elements. The action-current of the exciting element must have a duration sufficient to enable it to excite the responding element; it must also rise to its maximum with sufficient rapidity. Now rapid nerve-conduction means rapid local electrical variation, *i.e.*, brief duration of the current-strength requisite for stimulation. Only a rapidly responding muscle can thus be stimulated by the action-current of a rapidly conducting nerve; in the case of a slowly responding muscle the duration of the action-current of such a nerve would be insufficient to cause excitation. Similarly a slowly conducting nerve is unable to impart the requisite stimulus to a rapidly responding muscle, because the electrical variation in such a nerve, even if sufficiently intense, rises to its maximum too gradually to stimulate a tissue with a brief time-factor of excitation, although it might well be capable of stimulating one with a slower rate of response.[61] The correlation between the velocity of conduction and the velocity of contraction is thus not to be regarded simply as an adaptive arrangement which aims at securing quickness of reaction by shortening the total time of response, but as essentially an expression of the general validity of the fundamental laws of electrical excitation, namely, that the stimulating (*i.e.*, polarizing) current must have a certain minimal duration and a certain minimal rate of development as well as a certain intensity. A slowly conducting nerve is by its very nature one whose action-current is unable to stimulate a rapidly responding tissue, and *vice versa*.[62]

The chief objection to the present hypothesis seems to me to be that transmission may be blocked by local narcotization, or by mechanical constriction or local injury which affects only a short stretch of nerve. Such local alteration of course leaves electrical

[61] Compare with the examples of failure of secondary excitation quoted above from KÜHNE p. 424.

[62] Conceivably, of course, intermediaries — "transformers" — might exist which would render such transmission possible, but this possibility need not be considered here.

conductivity unimpaired. The conditions of many of these blocking effects are, however, imperfectly understood at present, and it does not appear that their existence is necessarily incompatible with the present view. In the case of ligature, severing, or the local application of a poison, there is local injury; this involves the production of demarcation-currents extending in both directions from the region of injury for some distance through the adjoining tissue; these currents not only exert electrotonic influence, but they may directly compensate the action-current of an approaching excitation-wave and so prevent its effect from extending beyond the point of injury.[63] The reversible blocking action of narcotics is more difficult to explain. The narcotized region must, however, have a certain length. Recent experiment shows that the extinction of the excitation-wave on entering a narcotized area is not an immediate, but a more or less gradual process. The excitation-wave may pass such a region if the latter is not too long.[64] Its length may, however, be considerably less than the 2 or 3 centimeters across which the electrical impulse from an active region is above supposed to be effective. It is possible here also that demarcation-currents interfere with the action-current and prevent its transmission across the narcotized area. I shall not attempt to discuss this difficulty further at present. The possibility that the

[63] It is known that pricking the *His* bundle so as to injure directly only a few fibres may completely block for a time the impulse from auricles to ventricles. For instances *cf.* ERLANGER and BLACKMANN: Heart, 1910, i, pp. 211, 214, 217. Similarly, partly cutting across a bridge of muscle (sufficiently wide to conduct regularly) connecting two portions of the turtle's ventricle may cause complete temporary block. This disappears in time on bathing in Ringer's solution. Then a further injury at or *near* the original cut will restore the block. (Observations of DR. W. E. GARREY; similar observations are contained in his article on fibrillation in the American Journal of Physiology, 1914, xxxiii, p. 397.) Some influence due to the injury of adjoining elements prevents the transmission of impulses along the uninjured elements. GARREY has also expressed the view that changes in potential at the site of block form a chief factor in the production of this effect (public lecture delivered at Woods Hole, July, 1913).

[64] *Cf.* ADRIAN: Journal of Physiology, 1912, xlv, p. 389. Also the papers of FRÖHLICH (Zeitschrift f. allg. Physiol. 1904, iii, p. 148) and others, showing a progressive decrease of impulses in narcotized regions or others conducting with a "decrement."

action-current is the actual normal basis of transmission, even in nerve, seems on the whole strongly favored by the facts and considerations cited above. That there are difficulties in extending this view in detail to special cases of transmission it would be useless to deny. But it is only by definitely formulating an hypothesis and tracing its consequences that we can decide as to its adequacy or inadequacy. Criticism and further research both are necessary before a final decision can be reached.

It is evident that according to the point of view urged in this paper the problem of physiological conduction ceases to be a special problem, and becomes a subordinate part of the general problem of stimulation, — more specifically of electrical stimulation. The idea that conduction and excitation are two separate and independently variable properties of an irritable tissue has shown itself inconsistent with the course of recent research and is now very generally rejected.[65] Why the electrical current should stimulate is the essential problem. The view that electrical stimulation is an expression or consequence of the production of an electrical polarization at the surfaces of the irritable elements was frequently urged by Hermann,[66] and has been placed on a firm footing by the work of Nernst and his successors. A further important step was made when Brünings[67] first pointed out what seems to be the essential implication of the law of polar stimulation, that the necessary antecedent for stimulation is a *depolarization* of the plasma membrane, *i.e.*, a polarization-effect in the inverse direction to the pre-existing or physiological polarization and hence having the effect of partly compensating this. The electrical current thus stimulates when it suddenly *decreases* the physiological polarization to a certain critical degree. To do this it must act for a certain time and rise to its maximum with sufficient velocity.

[65] *Cf.* FRÖHLICH: *loc. cit.;* also the discussion by LUCAS: Croonian Lecture, *loc. cit.;* and VERWORN: "Irritability," Yale University Press, 1913, chap. vi.

[66] *Cf.* HERMANN: Handbuch, Vol. ii, 1, p. 193 and elsewhere.

[67] *Cf.* BRÜNINGS: Archiv f. d. gesammte Physiologie, 1903, *c*, p. 367. HERMANN entertained similar views: *viz.*, that in nerve the stimulating action had its seat at the boundary of axis cylinder and sheath, and that a fibre is stimulated when the positive polarization of its longitudinal surface is decreased or its negative increased (*loc. cit.*, p. 193).

The essential question thus becomes: why should a slight decrease in the electrical polarization of the plasma membrane cause stimulation? Evidence from many sides indicates that during stimulation the plasma membrane temporarily loses its semi-permeability;[68] and since slight depolarization results in stimulation, the inference seems justified that the normal resting permeability of the membrane *depends* on its electrical polarization.[69] The view that the permeability of the plasma membrane is a function of the electrical polarization has also been independently urged by several other investigators.[70] The nature of the relation between the electrical polarization and the other properties of the membrane is still uncertain. During the normal resting condition of the irritable element the membrane appears to retain its semi-permeability unaltered; a sudden depolarization results in a change which has the effect of temporarily altering both its osmotic and its electromotor properties. Stimulation is apparently an expression or consequence of this change. At present the evidence indicates (1) that the change in permeability associated with stimulation is not a direct effect due to merely physical changes in the protoplasmic surface-layer, but is the consequence of a chemical reaction which alters the character of the surface-film and temporarily deprives it of its normal semi-permeable and electromotor properties, and (2) that this chemical process may in a highly irritable tissue like nerve be initiated by any slight local decrease in polarization provided the change is sufficiently rapid. That the process which is immediately initiated or released by the polarization-change is a *chemical* one is indicated both by the high temperature-coefficient of the stimulation-process, and by the wide variation which its

[68] I have summarized and discussed this evidence in various places: *Cf.* American Journal of Physiology, 1909, xxiv, p. 14; 1911, xxviii, p. 197; Science, 1909, p. 245; Biological Bulletin, 1909, xvii, p. 188.

[69] *Cf.* American Journal of Physiology, 1908, xxii, p. 79; Biological Bulletin, *loc. cit.*

[70] *Cf.* GIRARD: Journal de physiol. et pathol. gén., 1910, xii, p. 471; W. B. HARDY and H. W. HARVEY: Proc. Roy. Soc., Ser. B. 1911, lxxxiv, p. 217; HÖBER: Physikalische Chemie d. Zelle u. d. Gewebe (3d Ed.), 1911, p. 308 *seq.*; MINES: Journal of Physiology, 1910, xl, p. 309, 1912, xliii, p. 467; A. J. CLARK: *ibid.*, xlvii, p. 103.

velocity shows in different tissues.[71] The conditions suggest that the normal polarization is what prevents certain constituents of the surface-layer from interacting, some critical reaction being held in check so long as the polarization has a certain value — very much as the chemical interaction between plates and liquid in a battery with open circuit is held in check by the polarization at the surface of the plates. The sensitivity of highly irritable elements to variations in the electrical polarization of their surface-films is difficult to understand on any other hypothesis known to me at present.[72]

Summary

1. Numerous instances are cited from the literature showing the existence of a general parallelism between the rate at which the action-current curve rises to its maximum in any irritable tissue and the rate of propagation of the excitation-wave in that tissue.

2. Various other facts are cited favoring the hypothesis that the transmission of excitation from one region of an irritable tissue to another is directly due to electrical excitation of the inactive region, at some distance from the active region, by means of the portion of the action-current flowing between the two regions.

3. On this view the rate of propagation depends on two factors: (1) the time required for the action-current to rise to its full intensity after stimulation, and (2) the maximal distance from the active region at which the intensity and duration of the branch of current traversing the inactive region are sufficient for stimulation.

[71] Estimates of the relative velocities of the excitation-process in different irritable elements are given in the paper by Lucas; Journal of Physiology, 1910, xl, p. 225; also by Lapicque: Comptes rendus de la Société de Biologie, 1905, lvii, p. 503.

[72] Changes in the polarization of the plasma membrane must involve changes in the aggregation-state of the protoplasmic colloids (*cf.* my earlier paper *loc. cit.*, 1908), since, as is well known, colloids are sensitive to changes in the electrical polarization of the colloidal particles. The possibility that the primary change in stimulation may be one of this kind should thus also be considered. Undoubtedly some change in the aggregation-state of the colloids of the membrane is involved in stimulation, but whether this is primary or secondary cannot be said at present.

If the time occupied by the rise of the action-current to the critical stimulating point is t seconds and the maximal distance at which it causes stimulation is s centimeters, the velocity of propagation will be s/t centimeters per second.

4. In frog's nerve the distance s is estimated at from 2 to 3 centimeters and the time t as .001 second (the duration of the rising phase of the bioelectric variation at 18°); this corresponds to a velocity of 20 to 30 meters per second at this temperature. Increase of propagation-velocity with rise of temperature appears to be due chiefly to the increased rate of rise of the action-current; at 38°, with $Q_{10} = 2$, it would be 80 to 120 meters per second.

NOTE UPON THE MOVEMENTS OF THE INTESTINAL VILLI

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THE structure of the villi of the small intestine is such that one would naturally infer that they possess the property of motion. Accordingly observations were begun on the living animal to see if such is the case. The dog was selected for these experiments.

The technique employed in performing the experiments is as follows:

The dog is anaesthetized with ether and the abdomen opened in the median line. A loop of the small intestine is picked up and passed through a slit in a thin board, or a slab of hard paraffin. The intestine is opened longitudinally for a distance of two or three inches opposite to the attachment of the mesentery and spread out and fastened to the board by means of tacks inserted into the board on each side of the slit, care being taken that the mesentery is not stretched nor pressed upon so as to interfere with the circulation or nerve supply.

The observations are then made by means of the binocular microscope, using a magnification of from 23 to 61 diameters. In dogs that have been fasting from 24 to 48 hours the villi are generally found extended, fallen over in various directions, and covered with a mucus-like substance. Local application of a few drops of any of the following solutions: — peptone, glucose, weak alkali solutions (sodium carbonate), dog's bile, diluted, physiological salt solution, or distilled water, is followed in a few seconds by the villi rising and beginning lashing-like movements in various directions. Very soon a second distinct movement begins which

consists of an alternating shortening or retraction, and extension of various villi in the field. These movements are independent of peristalsis. The application of hydrochloric acid, $\frac{1}{10}$ per cent solution, checks the movements, and the villi return to the resting condition, i.e., fall over and soon become covered with mucus. A 10 per cent alcoholic solution first stimulates, then depresses, and soon stops the movements entirely. The addition of water, or saline solution, 0.9 per cent, restores activity and the villi begin movements again. Intravenous injection of nicotine (1 mg. to 2 mg. per kilo) first stimulates, then depresses the movements, particularly that of retraction and of extension. Local application of solutions that caused active movements before the injection of the nicotine now have no effect. Atropine (1 mg. per kilo, intravenously) stops the movements. Local application of glucose, peptone, or other solutions that stimulated previous to the use of the atropine cause the lashing-like movement to return, but there is no retraction and extension.

From the observations thus far made, which have been quite constant on all animals examined, we reach the following conclusions:

First. That the villi possess distinct movements that are independent of peristalsis.

Second. That these movements are of two kinds: (1) A lashing movement, which may be supposed to aid in the mixing of the intestinal contents and thus promote the action of the digestive secretions as well as the process of absorption. This movement is not stopped by atropine. (2) An alternating retraction and extension, a form of movement which may be of special value in the act of absorption, particularly the absorption through the lacteals. This movement is abolished by both nicotine and atropine and is therefore under the control of the peripheral nervous mechanism. It may be regarded, probably, as a local reflex. Further investigation is necessary to determine whether the central nervous system is involved in this reflex.